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SYMPOSIUM ON SPECIES AND CHROMOSOMES[1]

SPECIES AND CHROMOSOMES

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IT is only since the beginning of the present century that the progress of biology has rendered possible the discussion of this topic. When the "Origin of Species" was written, chromosomes were unknown, and the study of nuclear division and the relation of chromosomes to the life cycle only began in the seventies and eighties of the last century. The names of Flemming, Strasburger, Boveri and many others are connected with the early work on chromosomes, when alcoholic fixation and methods of sectioning first made possible their study.

In the last fifteen years particularly, the alliance between cytology and genetics in the experimental study of heredity and variation has become increasingly intimate. Beginning with the recognition of chromosome segregation in meiosis as the basis of Mendel's law of heredity, this was soon followed by the discovery (1906) of definite relations between chromosome numbers and

[1] At the request of the Sectional Committee having the discussion in charge, Professor E. C. Jeffrey has secured from their authors most of the contributions to the discussion on "Species and Chromosomes," which were given at the Toronto meeting of the British Association for the Advancement of Science. There has been some delay on account of the illness of two of the most important contributors and one of them is still too infirm to have supplied as yet a summary of his remarks. It will be noted by the reader who is familiar with the names appearing in connection with the symposium that Mr. Julian Huxley and Dr. Heslop Harrison, two zoological contributors, are not represented. Mr. Huxley was absent from the discussion and indisposition has prevented Dr. Harrison's paper arriving in time for inclusion in the symposium.

mutations in Oenothera. Since 1910 Morgan and his colleagues have built up a combined system of genetical and cytological results in Drosophila which is unequalled in any other organism. Their theory of linkage and crossing-over has brought the phenomena of meiosis, chromosome structure and heredity into still closer relationship More recently Blakeslee and Belling have discovered chromosome relationships in Datura which parallel many of those formerly obtained with Oenothera, and in a striking series of experiments have extended those results in various directions.

In recent years, a number of predictions of peculiar cytological conditions have been made on the basis of genetic experiments, and afterwards confirmed by observation of the cells. An example is the work of Bridges with strains of Drosophila having an extra chromosome. This condition arises, as was first shown in Oenothera, through failure of one pair of chromosomes to enter separate nuclei in reduction, a process which is now known as non-disjunction.

The following may now be regarded as generally accepted facts concerning the chromosomes. They are bodies of relatively fixed size, shape and point of attachment to the spindle fibers. Homologous pairs usually separate in meiosis and thus furnish the basis for simple Mendelian behavior. Their number is generally fixed for each species so far as the germ-lineage is concerned. The relations of genetical crossing-over to chromatin behavior are complicated and need not concern us here Aberrant chromosome numbers arise in various ways and produce forms with different external characters Such forms have been specially investigated in Oenothera, Drosophila and Datura. The most striking changes found are tetraploidy ($4n$ chromosomes), triploidy ($3n$) and non-disjunction leading to forms with an extra chromosome ($2n+1$, $4n+1$, etc.). These conditions occur in all three genera, except that tetraploid forms have not been observed in Drosophila. The con

dition found in Oenothera particularly furnished the first experimental basis for a comparison of the chromosome numbers in various wild and cultivated species of plants.

When we examine the chromosomes of related species of plants and animals, differences in number or in size and shape of the chromosomes are often found. How have these differences originated? The answer to this question has already given us the beginnings of a chromosome phylogeny, which can now be soundly based on analogy with experimental results. The case of *Oenothera gigas* furnished the first experimental clue to the origin of tetraploidy. Many wild tetraploid species of plants are now known, and they must have arisen in some such way as *Oe. gigas* appeared, *i.e.*, as mutations. The tetraploid and higher polyploid species are turning out to be surprisingly common in plant genera, though apparently much less frequent in animals.

Let us attempt to classify the various types of change which are indicated to have taken place in the phylogeny of chromosomes by the comparison of related species or genera. We may mention:

(1) Polyploidy, or the development of higher multiples of chromosome numbers.

(2) Transverse segmentation or fragmentation of all the chromosomes, or of particular pairs. A phenomenon of this nature seems to have occurred on a large scale in the phylogeny of the Liliaceae.

(3) The end-to-end fusion of certain chromosomes. This is particularly clear, both from genetical and cytological evidence in certain species of Drosophila, and appears to have happened in various other cases.

(4) Irregular division or non-disjunction may lead to forms with additional pairs of chromosomes.

(5) The recent genetic evidence from Drosophila and the behavior of the chromatin in such forms as *Oenothera lata* indicate that not only may chromatin fragments enter a nucleus and afterwards divide, but also that portions from one chromosome may become attached to an-

other and thus alter the genetic relationships of the factors concerned. This process is one which must also be considered as important in the evolution of species.

(6) In the genus Carex, Heilborn (1924) has shown that the haploid numbers in forty-four species run from 9 almost continuously to 42 and 56, but without multiple numbers. Heilborn suggests that one of the most important methods of chromosome increase in Carex has been by mutations of the *Oenothera lata* type followed by a division of the extra chromosome.

(7) Certain species and even genera with new chromosome content have probably arisen through crossing. Cultivated maize may have arisen from a cross between the Mexican Euchlaena and some species of grass belonging to the Andropogoneae. J. Clausen finds that *Viola tricolor* and *V. arvensis* have, respectively, 13 and 17 chromosomes as haploid numbers. In a cross the F_1 plants have 30 chromosomes, but in certain of the pollen mother cells 47 were found, *i.e.*, $13 + 17 + 17$, a condition of semi-tetraploidy.

It is thus becoming clear that chromosome changes have arisen in a variety of ways, including transverse segmentation or fragmentation, end-to-end union of certain pairs, gradual diminution and disappearance of certain chromosomes, non-disjunction, rearrangement of portions of chromosomes, crossing of species having different numbers and polyploidy.

From the work of the last fifteen years, it has become clear that polyploidy has played an important part in the evolution of many plant genera and families, and that it is of particularly common occurrence among the varieties of cultivated plants. If tetraploidy can be brought about, as seems likely, by a lowering of temperature when the fertilized egg is beginning to divide, then one may perhaps understand its prevalence in plants and its relative infrequency in animals where temperatures are more controlled. But that it can be produced in other ways is shown by its experimental

production in mosses by wounding (Marchals), in Solanum by grafting (Winkler), and semi-tetraploidy at least by crossing.

The fact that such notoriously polymorphic genera as Rosa, Rubus and Crataegus are all found to show polyploidy in a high degree is a discovery too fresh for its significance to be fully realized.

Similarly in the evolution of various other genera and families, higher and higher polyploid numbers have been reached. Thus in the genera Chrysanthemum, Rumex and Rosa, species are found which are $2n$, $4n$, $6n$ and $8n$ in their chromosome constitution. Some of these genera also have $3n$, $5n$ and even $10n$ species. Many of these species are apogamous, and where the chromosome number is an unbalanced one, as in $3n$ and $5n$ species, it can only be maintained by apogamous or vegetative reproduction. Thus these unbalanced species must always have been apogamous from their origin.

The conditions in the wheats, where $n=7$, may be briefly mentioned, Einkorn wheat (*Triticum monococcum*) is diploid, the species of hard wheat are tetraploid, and the Vulgare wheats (soft), which comprise our highest yielding modern sorts, are hexaploid. When members of different groups are intercrossed they produce sterile hybrids. All three groups go back to prehistoric times. The tetraploid wheats presumably arose, like *Oenothera gigas,* through a sudden doubling in the number of chromosomes. The most probable hypothesis of the origin of the $6n$ condition is that it came from a $3n$ hybrid between diploid and tetraploid species, one such hybrid having doubled its chromosomes, perhaps in the fertilized egg, and so restored its fertility. In certain cultivated varieties of mulberry, the $3n$ condition is maintained by apogamy, and the same is true of the wild species *Eupatorium glandulosum* and *Erigeron annus.* Similar polyploid conditions are being found in many cultivated plants, including bananas, pineapples, oats and dahlias, so that much more evidence concerning the

precise manner of origin of these conditions ought to be obtainable.[2] Enough is already known to show that several different kinds of, for example, tetraploidy exist.

The development of sterility between species has undoubtedly played an important part in evolution. How this condition has come about is still a matter of speculation, but we are beginning to get some light on the subject. The old view that forms which produced fertile hybrids must belong to the same species has completely broken down, as has the converse conception that in plants sterile pollen is a proof of hybridity. One of the most interesting features of tetraploidy is its relation to the development of sterility. As has been pointed out elsewhere,[3] if a tetraploid form such as *Oenothera gigas* arises in nature, its hybrids with its diploid parent must contain an unbalanced chromosome content, and this leads to the dropping out of chromosomes in later generations until the diploid number is restored.[4] The triploid form can only perpetuate itself by developing apogamy (as certain triploid species and varieties have done) or by doubling its chromosomes, thus producing a hexaploid form. In all three cases new centers of stability will be reached, which are starting points for fresh variations. The essential conditions for physiological isolation are produced.

In this way tetraploidy and the higher forms of polyploidy, as well as other types of chromosome change, have been of great importance in evolution, since they have the effect of physiological isolation even when there is no isolation in space.

The study of chromosomes thus leads to results of far-reaching importance for evolution. It is of the highest importance for taxonomy in determining the relationships of species and genera. With advancing knowledge

[2] Some of these subjects have been more fully discussed elsewhere. Polyploidy, *Brit. Journ. Exptl. Biol.*, I, 153–182, 1924.

[3] *Nature*, 1922, vol. 110, pp. 179, 447.

[4] See Gates, "The chromosomes of a triploid Oenothera hybrid," *Ann. Bot.*, 37, 565–569, 1923.

and quicker methods of examining chromosomes, the time should come when the description of a species is not considered complete until the morphology of its chromosome group is known, for visible chromosome differences, when they occur, throw important light on relationships. With further knowledge we shall doubtless learn much concerning the phylogenetic nature of the various kinds of chromosome change which have been outlined here. They suffice to indicate that there is a fundamental evolution of the germplasm, of which changes in specific characters are merely the external expression. Already as regards tetraploidy these changes have been experimentally analyzed and we know something of the conditions which bring them about.

Modern genetics has led us to deal, as Bateson has said, with the world of gametes which form the background for the visible inherited variations of species. The next decade of comparative and experimental work with chromosomes will bring the realization that in the secular changes of the chromosomes, however controlled or determined, we have a means of understanding how the cell unit, and through it the organism, has varied in structure in the past from species to species. This gives a new line of approach, of fundamental importance, in the analysis of the processes of evolution. It is to be hoped that its significance will be recognized, not only by experimental evolutionists as an additional line of evidence in tracing phylogenies, but also by taxonomists as a necessary element in their discrimination of genera and species.

From a phylogenetic point of view, two important principles have emerged from the work of recent years in experimental evolution and other fields. One of these is the principle of parallel mutations, which introduces a new element of fundamental importance into the reconstruction of phylogenies. The original case of parallel mutations experimentally determined was that of the *lata* mutations from *Oenothera Lamarckiana* and from

Oe. biennis, both mutants having 15 chromosomes and the same peculiarities of leaf and habit and sterile pollen, but each having the flower-size of the species from which it was derived. Innumerable cases of parallel variations are now known in plants, and many in animals. They appear to have originated as mutations. When their significance is appreciated by systematists they will have a profound effect on present conceptions of relationships and phylogeny, for they show that many similar variations have occurred over and over again in unrelated groups.

The other principle has been emphasized particularly by paleontologists in recent years.[5] We may call it *orthophylesis* to avoid the special connotations of previous terms. It consists in the recognition of longer parallel phylogenetic trends in different lines of descent. What relation, if any, these orthophyletic trends bear to parallel mutations remains to be investigated. But it is possible that some of them, at any rate, as pointed out earlier in this paper, may be the result of changes initiated in the chromosomes. In any case the comparative study of chromosomes offers one of the most promising lines of advance in experimental evolution.

CHROMOSOMES AND CLASSIFICATION IN THE GENUS *ROSA*

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ROSES provide such a tantalizing array of forms to the taxonomist that it seemed a promising group on which to test the possibility of throwing light on classification by means of a study of cytological conditions. The results have amply rewarded the effort; for the work of Täckholm (4, 5) and of Heslop Harrison and myself (2) has shown that this line of research is capable of yielding

[5] See Watson, D. M. S., Croonian Lecture, Royal Society, 1924 (*Nature,* June 7, p. 841).

very useful data both from a taxonomic and a general evolutionary aspect.

In *Rosa* the fundamental chromosome number is seven and the majority of American forms have this as the haploid figure, being therefore diploid; among these are *R. carolina, R. nitida* and *R. setigera.* In the British Isles the only native diploid form is *R. arvensis.* Again in England we have only one tetraploid form, *R. pimpinellifolia,* whereas in America there are *R. blanda, R. lucida, R. pratincola* and other forms. The remaining American forms have twenty-one as their haploid number and are hexaploid. Thus the western species provide among themselves a good polyploid series with diploid, tetraploid and hexaploid forms. Such series have been described in other genera, but the greatest cytological interest is focussed on the eastern group, Caninae, to which most of our English species belong. This section is one of the greatest puzzles to the taxonomist, since the innumerable forms run into one another to such an extent that it is well-nigh impossible to define any species limits at all. For this reason we examined the forms cytologically as well as in the field to try to throw further light on the subject. The following is a brief account of the results obtained which are in some respects unique with regard to cytological behavior.

To illustrate this we may take for example a form from the sub-section Villosae, *R. mollis* will serve: Its somatic chromosome number is twenty-eight, exactly as in *R. blanda* or *R. pimpinellifolia* and it is therefore tetraploid. In the reduction division in the anthers the early stages show nothing to arouse suspicion that there is anything abnormal, but at the metaphase the chromosomes are very slow to arrange themselves on the equatorial plate and, when a particularly tidy plate enables the chromosomes to be counted, it is seen that, instead of fourteen, as might have been expected, there are twenty-one. To cut a long story short it was found that the central seven of these, which are larger, are bivalents, and the pe-

ripheral fourteen univalents. The bivalents promptly separate and pass neatly to the poles, but the univalents divide and follow them more slowly; and so, when the daughter nuclei are formed, many of the chromosomes are left out. These later form micronuclei. The same story is repeated in the next division, which is even more irregular. Finally up to eight pollen grains may be formed instead of a normal tetrad and most of these are abortive; only those which contain the seven chromosomes descended from the bivalents appear to survive and probably those with exactly seven are the most fertile.

The other subsections of the Caninae examined showed similar behavior except that the somatic count is thirty-five. Such a pentaploid species shows itself to be abnormal even in its somatic cells, since the chromosome count is not, as usual, divisible by two. The reduction division in the anther again shows seven pairs of chromosomes and this time twenty-one univalents.

In addition Täckholm (5) has described one or two scattered varieties in which the somatic figure is forty-two and there are again seven bivalents with twenty-eight univalents.

Considering the features shown by these forms—the only partial reduction in the heterotype division, the formation of micronuclei and later of extra pollen grains and the generally bad state of the pollen—we are irresistibly drawn to a comparison with known hybrids with parents of unequal chromosome numbers such as appeared in Rosenberg's classical work on *Drosera anglica* (3). Such hybrids are usually unfertile, though there are exceptions to this. Why then do the Caninae set good seed and persist as such a dominant race? The answer to this, I think, that they have adapted themselves to their peculiar condition in two ways.

(a) Nearly all the forms referred to, if not quite, can reproduce themselves apomictically, that is to say, they produce purely matroclinous plants by some method independent of fertilization and so perpetuate the race.

(b) Megaspore development is quite different from microspore development, as Täckholm has shown, in that the fertile megaspore normally retains all the univalents and seven units from the bivalents, so that, should the plant be fertilized by one of the seven chromosomed pollen grains, the fertilized egg would contain exactly the chromosome complement of the parent. Our investigation of the hybrid *R. coriifolia* × *R. lutetiana* has shown that this actually takes place at times, extraordinary as it may seem.

Additional support to the hypothesis that these ordinary members of the British Flora really are hybrid in origin may be obtained by examining the cytology of some of the known rose hybrids. In those with both parents diploid, such as *R. cinnamomea* × *R. rugosa,* or both parents tetraploid, as in *R. pendulina* × *R. pimpinellifolia,* the behavior is regular at the reduction division and the plants are fertile, but cross *R. cinnamomea* with *R. pendulina* and you obtain a triploid individual which, at the reduction division, gives seven bivalents and seven univalents in just the same way as in the Caninae forms described above. Again *R. pendulina* × *R. nutkana* has 14 + 21 chromosomes, of which fourteen pair at the reduction division in a similar way.

We have thus in the roses a group of plants with a base number of seven chromosomes in which there are several polyploid series: the first series comprises normally reducing forms with their somatic chromosomes in multiples of fourteen giving thus diploid, tetraploid, hexaploid and even, in one type of *R. acicularis,* octoploid forms.

The remainder of the roses have partial reduction only and fall into two groups according to the number of bivalents at the reduction division. The one series with seven bivalents contains forms which are triploid, tetraploid, pentaploid and hexaploid, and the second, with fourteen bivalents, contains pentaploids and hexaploids.

We have now to consider the question of the possible origin of these different chromosome complements, which

is, in this case at any rate, practically the same question as the origin of the species.

The Caninae section has been shown to be probably derived by hybridization at a relatively recent date but, for the origin of its various forms, it is necessary to assume an original polyploid series with forms up to the octoploid.

It needs to be considered if we have any evidence as to a possible method by which the original polyploid might have arisen from diploid ancestors. I shall proceed to show that some evidence is now available (2).

If you consider the possible crosses between a member of the section Caninae and a rose of some other section, since the pollen and embryo-sac of the former have a different complement of chromosomes it will be obvious that reciprocal crosses might be expected to have a different chromosome number.

Consider the hybrid *R. tomentosa* $\times$ *R. pimpinellifolia,* which is a not uncommon combination in the British Isles: if *R. tomentosa* is the female parent the chromosome complement should be $28 + 14 = 42$ and the plant will be strongly matroclinous. Such a hybrid we know in *R. Sabini,* which behaves exactly as might have been expected in its reduction division, resembling *R. tomentosa* except for the addition of seven chromosomes giving fourteen bivalents. This form is quite sterile, the flowers drop off at an early stage.

A search for a reciprocal hybrid among naturally occurring plants revealed *R. Wilsoni* growing at Bangor, N. Wales. Miss A. J. Davey, M.Sc., very kindly prepared for us slides of this form and we were thus able to examine its cytology. We expected to find $14 + 7 = 21$ chromosomes, since we have assumed that pollen grains with seven chromosomes are most likely to be fertile. To our great surprise we found that it possesses 42 somatic chromosomes and moreover shows almost no evidence of hybrid nature in its cytology. Its reduction division is almost like that of such a normal hexaploid

form as *R. nutkana,* and moreover it sets good seed. Now had the parents possessed an equal number of chromosomes this might not have been surprising; as it is, some explanation is required. Carefully considering all possible hypotheses, we were drawn to the conclusion that the chromosome number had doubled, probably by a suspended division in the egg cell, and thereby given each chromosome a synaptic mate. The fertility is possibly dependent on this last factor.

If this hybrid has become fertile by doubling its chromosomes it suggests a possible origin for all the known polyploid forms in the genus; the balanced ones arising by hybridity and doubling, and later the unbalanced ones by hybridity followed by apomyxis.

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CHROMOSOMES AND SPECIES

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THE problem of the systematic value of the chromosomes, their number and shape, is a rather new one and it is hardly possible to make any general statements. But as Tischler and others have shown, a study of the chromosomes in smaller systematic groups may already be used for the elucidation of relationships.

From a cytological point of view pure species are characterized by a certain balanced diploid number of chro-

mosomes. An analysis of the mode of formation of such balanced numbers will surely give some explanation of the importance of the relation: chromosomes and species.

In many cases new species may arise by new combination of genes, and one might then be justified in concluding that sometimes, by adding or combining new chromosomes to another balanced number, a new constant type would result.

Hybridizing may give new constant chromosome-combinations as a result. However, in the case of the *Drosera*-hybrid, a cross between two gametes, differing in number of chromosomes, the result was an offspring which in the heterotypic division gave nuclei with a very different number of chromosomes. In *Papaver* we have a very interesting case where crossing resulted in an increase in the balanced number of chromosomes. Miss Ljungdahl crossed two species with 7 and 35 chromosomes, respectively, in the gametes. The hybrid had 42 as diploid number, and 21 gemini were formed in the heterotypic division; back-cross to the one parent with 7 chromosomes gave an offspring with 28 chromosomes in the somatic division, and 14 gemini in the heterotypic division. Thus, the crossing-experiments gave as result types with a new balanced number of chromosomes; we may say from a cytological point of view, new species. The constancy in chromosome number is caused by allosyndesis, *i.e.*, conjugation between homologous parent-chromosomes and autosyndesis, which means conjugation between sets of chromosomes belonging to the same parent. Accordingly, we see that the pairing of chromosomes is determined by the degree of homology as well as by the descent from sperm and egg.

In the case of non-disjunction in the heterotypic division the two non-conjugating chromosomes may behave in different manners:

(1) They may both go to the same pole, and, as regards the number of chromosomes, the result will be that

after the homotypic division the number of chromosomes will be higher in two tetrad-cells and smaller in the other two; (2) if the non-conjugating or single chromosomes in the metaphase are situated in the equator they will often split longitudinally in the anaphase and consequently the result will be an increase in number of chromosomes in the 4 tetrad-cells; (3) sometimes these single longitudinally split chromosomes are situated nearer one pole in the anaphase, and will be included in the daughter nucleus at interkinesis. In this case extra chromosomes are added to one of the daughter nuclei.

These irregularities refer to the first or heterotypic division. In the second, or homotypic one, the chromosomes split longitudinally, and the same irregularities as regards the single chromosomes often occur. The single chromosomes which split longitudinally in the first division will often split again in the second one, but a little later than the gemini chromosomes, thus causing delayed chromosomes. Accordingly, the number of chromosomes in the gametes must vary. In one *Hieracium* species with the somatic number 36 I counted from 15 to 25 chromosomes in the pollen-cells after reduction division with non-disjunction. One should have expected only 18.

After fertilization the offspring will form individuals with a very different number of chromosomes. In most cases perhaps the number of gemini in the heterotypic division will be the same as in the parent-individuals, with or without single chromosomes. However, in other cases the fertilizing gametes may have some single chromosomes homologous. The result will be that in the heterotypic division the homologous singles pair to gemini, and the number of gemini, *i.e.,* the balanced chromosome-number, will increase.

In some crossing-experiments with species of the genus *Hieracium* I have found such an increase in number of gemini realized. The female plant was itself a hybrid with 42 chromosomes, 36 of which paired to 18 gemini—

in the following called gemini-chromosomes—and 6 single ones in the heterotypic division. The male-parent had typically 18 gemini and some singles. Nevertheless, in the pollen-cells I found the chromosome-number varying from 15 to 25. In the F_1-offspring in some individuals I counted 25 gemini and some singles—this increase probably being due to a pairing of homologous single chromosomes in the gametes. In one hybrid between two *Hieracium*-species, which normally have 18 gemini, but also non-disjunction, I counted 33 chromosomes, and in the heterotypical division 15 gemini and 3 unpaired chromosomes. Probably the pollen- and egg-cells of the parent-species had 18 to 15 chromosomes. Hence, by non-disjunction we have obtained a type with a lower balanced number than the parent plants, thus forming a cytological basis for a new species.

A very interesting problem has turned up lately in consequence of recent years' very increased knowledge on the number of chromosomes in related species and genera: Is there any relation between the number of chromosomes and the phylogeny of the species?

One has recently tried to support the theory that in small groups species with the higher chromosome-number should represent the younger types; phylogenetical lines should go from groups with low number to those with higher ones. According to my opinion it is very difficult to make any decision yet. I am inclined to think that in small systematic groups higher numbers are derived from the lower ones. But this does not mean that a species with a small number must therefore be considered as a more primitive species than another one from the same group, but with a higher number. And in fact, it is not yet quite clear if not a higher chromosome number in some cases could be acquired by transverse section of chromosomes; if so, one might also expect that chromosomes could fuse end to end, which would result in a decrease in chromosome number.

POLYPLOIDY AND THE ORIGIN OF SPECIES

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IT is known as a result of the investigations of recent years that frequently different species of the same genus may manifest diverse equipments of chromosomes in their cell divisions. For example, the species of Chrysanthemum, according to Japanese investigations, may exhibit 9, 18, 27, 36 and 45 in their reproductive mitoses and their sporophytic cell-divisions which represent the double of the gametic numbers are as a consequence known as diploid, tetraploid, hexaploid, octoploid and pentaploid. In addition to this type of polyploidy there is another, well exemplified by large genera of the Rosaceae. In the Rosaceae, as a result of the very interesting investigations of Rosenberg and his student, Täckholm, in Sweden, of Blackburn and Harrison in England, and of Longley in America, we know of the existence not only of diploid, tetraploid, hexaploid and octoploid species but also of species which are triploid and pentaploid. In the gametophytic mitoses of the triploid and pentaploid species complications are introduced by the fact that the sporophytic number of chromosomes when divided by two does not result in an even multiple of the gametophytic number. Since it is apparently desirable to have names to describe these two types of polyploidy for the even multiples, as in Rosa and other genera, the term artioploid may be suggested, and for the uneven multiples the corresponding vocable perissoploid.

Before passing on to the consideration of the relation of polyploidy, artioploidy and perissoploidy to the origin of species, it will be well to refer to still another type of abnormality in chromosome equipment, which is presented by plants. A notable illustration of this condition is furnished by the very recently published investigations of Heilborn on the huge sedge genus Carex. Here the numbers of chromosomes in mitosis varies greatly

and does not come under the law of multiples. The numbers, for example, run 9, 15, 16, 19, 24, 25, 26, 27, 28, 29, 31, 32, 33, 34, 35, 36, 37, 38, 40, 41, 42 and 56.

A similar state of affairs is found in the compositaceous genera Lactuca and Crepis as well as in other instances, which need not be mentioned here, since we are merely exemplifying general conditions. The non-multiploid variation in number of the nuclear chromosomes may advantageously be termed dysploidy, since it represents variations in number which are irregular and do not conform to any definite numerical principle. Dysploidy is a phenomenon in general much less common than artioploid or perissoploid polyploidy.

In contrast to artioploidy, perissoploidy and dysploidy as defined above, we have many species which agree absolutely in their chromosome equipment; this for example is true of the genera Pinus and Lilium, which in general have the same number of chromosomes in their various species. In the genus Oenothera the number of chromosomes is usually seven (gametophytic) but variations from this condition are known in the case of certain of the so-called mutants or elementary species, where the chromosome equipment may be artioploid, perissoploid or dysploid in accordance with the terminology used above. It is the outstanding service of DeVries to have called the attention of biologists to these so-called mutants. The explanation of their origin is as yet unsettled, but resolution of this problem seems now measurably in sight. It will be clear, I hope, from what has already been said that in genera and species which are somewhat uniform in their chromosome equipment, variations from the normal may sometimes be found, which come conveniently under the headings perissoploid, artioploid or dysploid or even all these. These departures from the typical condition may conveniently be united under the general term polyploidy, although that vocable must then be used in a somewhat broader sense than is usual. Using polyploidy in the sense above de-

fined we have definitely reached the problem which falls to my share in the present symposium. There seems to be no doubt that polyploidy in the broader sense defined above is in certain cases definitely correlated to the phenomenon of the appearance of new species. The problem before us is to attempt to explain the origin of polyploidy in terms of known facts and thus the origin of certain kinds of species, not only of the so-called mutants or elementary species, but also of many of those species commonly recognized by the systematist.

It has been a common practice during the past two decades to cover our ignorance of the origin of polyploidy and associated phenomena by the use of the term mutation. The huge experimental activities in the case of Oenothera and Drosophila have to my mind been much less fruitful for our science than they would otherwise have been, on account of the often too limited point of view of mutationists. A great soldier more than a hundred years ago was wont to remark that God was on the side of the big battalions. Without wishing to endorse entirely that unmoral sentiment, I believe that it is beyond question that the study of large genera along both comparative and experimental lines is more likely to throw real light on certain modes of origin of species than has the intensive and extensive investigation of any of the so-called mutating species of Oenothera, Drosophila and Datura.

Since the general subject before us this morning is large and my time is limited, I shall focus attention on the Rosaceae as represented by the genera Rosa, Rubus and Crataegus. These are all large and polymorphic genera and exemplify in high degree the phenomenon of so-called mutation. It is clear from the study of experimentally produced hybrids that all the types of polyploidy may result from hybridization, namely, perissiploidy, artioploidy and dysploidy. Since the species of the Rosaceae often present the morphological characteristics of hybrids, it is reasonable to assume that to a very

large extent the origin of species, in this large order, has taken place as the result of the crossing of formerly existing species. This hypothesis gains strong support from the surprising ease with which hybrids are produced among the Rosaceae, both in nature and under experimental conditions. For these obvious and recent crosses, I have earlier suggested that the use of the term phenhybrid and for those older hybrids, to which the systematist dignifies with specific names, I have proposed the appellation crypthybrid. The work carried on in Professor Rosenberg's laboratory and in my own on the Rosaceae furnishes ample evidence of the hybrid origin of many recognized species. Recently the cytological investigations of Dr. A. E. Longley, carried out on the rich material growing in the Arnold Arboretum of Harvard University, make it clear that many species of Rubus and Crataegus are either phenhybrids or crypthybrids. There is, however, one direction in which question of the validity of these conclusions is likely to arise. Frequently on occasions such as the present geneticists have expressed themselves in an uncomplimentary manner regarding the value of morphology in evolutionary investigations. Although it is often evident that criticisms of this sort reveal some narrowness of outlook and even superficiality on the part of their authors, it will be well in this connection not to base our conclusions on morphological evidence alone, although this is by far the most utilizable and widely serviceable, in reaching general results regarding the origin of species, preeminently a problem for the morphologist.

The genus Rubus seems to be of very special importance at the present time, from the standpoint of the study of the origin of species in the Rosaceae. Professor Hoar, after an extended study of the organization of the pollen in the various American species belonging to the genus, particularly the Eubatus or Blackberry section, came to the conclusion that the species of Rubus were very largely of heterozygous or hybrid origin.

Some years later Brainerd and Peitersen, as the result of the systematic study of the blackberries of New England, came to the conclusion that there are many natural hybrids between recognized species of Rubus—these not infrequently in cultures, segregated back to their suspected parental forms. These hybrid or phenhybrid species are illustrated by *Rubus andrewsianus, R. orarius, R. frondisentis, R. glandicaulis, R. flavianus, R. fraternus, R. abbrevians, R. jeckylanus, R. vermontanus, R. viridifolius, R. junceus, R. groutianus, R. arenicola, R. alleghaniensis, R. argutus, R. pergratus, R. frondosus, spinus,* and *R. plicatifolius.* These authors recognize as more or less good species of Rubus the following: *R. alleghaniensis, R. argutus, R. pregratus, R. frondosus, R. canadensis, R. elegantulus, R. vermontanus, R. setosus, R. hispidus, R. flagellaris, R. baileyanus* and *R. enselenii.* Even in the last group, however, the pollen is often up to ninety per cent. sterile and the seedlings frequently show a considerable degree of variability, although in general true to the specific type. Apparently on systematic grounds some even of the second group must accordingly be regarded as crypthybrids.

In a second article appearing under the name of the junior of the two authors cited in the preceding paragraph, the pollen conditions in a number of Rubi are described. A greater or less degree of sterility marks all except *R. alleghaniensis.* The examination of the pollen was supplemented by germination experiments in which the results corresponded very closely with those derived from the morphological study of the pollen. In this and the preceding article the authors note the frequent Mendelian segregation of the seedlings of suspected hybrid forms towards their parents. For example, *R. andrewsianus* segregates to *R. alleghaniensis* and *R. argutus; R. arenicola* to *R. baileyanus* and *R. frondosus; R. cubitans* to *R. hispidus* and *R. vermontanus; R. flavianus* to *R. alleghaniensis* and *R. elegantulus; R. groutianus* to *R. elegantulus* and *R. setosus; R. jacens* to *R. hispidus* and

R. setosus; R. junceus to *R. canadensis* and *R. setosus; R. orarius* to *R. alleghaniensis* and *R. canadensis; R. permixtus* to *R. alleghaniensis* and *R. hispidus* and *R. trifrons* to *R. hispidus* and *R. setosus.*

The cytological investigations of Longley on the genus Rubus show a very interesting agreement with the general systematic and genetical results obtained by Brainerd and Peitersen. For example, the specific constancy of *R. alleghaniensis* from the systematic standpoint and its good pollen have a striking parallel in its cytological behavior, for in this species the gametophytic chromosomes are seven and there are no abnormalities in the heterotypic and homotypic mitoses, such as are often found in known hybrids. *R. argutus Link* comes within the same category, for a high degree of fertility, as exemplified by good pollen, has its counterpart in a diploid condition of the sporophyte and in regular gametic mitoses.

When attention is directed to the highly inconstant and largely sterile species which Brainerd and Peitersen on the basis of their Mendelian segregation consider to be of hybrid origin, the situation becomes even clearer. For example, *R. andrewsianus* Blanchard, which is considered by Brainerd and Peitersen to be a hybrid between *R. alleghaniensis* and *R. argutus,* both good diploid species, with normal generative mitoses, we find a triploid form similar to that derived by the crossing of diploid Oenotheras or diploid species or varieties of Morus. Poorly developed pollen and a meiotic mitosis resembling that of known hybrids are both exemplified by this obviously hybrid species. In *R. jeckylanus* Blanchard, which Brainerd and Peitersen consider to be a cross between *R. argutus* and *R. frondosus,* together with bad pollen, we find hexaploidy and irregular mitoses such as are typical of hybrids. The same statement stands for *R. frondosus* Bigelow; *R. plicatifolius* Blanchard, a hybrid between *R. flagellaris* and *R. frondosus,* according to the results of Brainerd and Peitersen, is

pentaploid and with this polyploid outfit of chromosomes unites a high degree of sterility and hybrid-like heterotypic and homotypic divisions. The same statement holds true for *R. hispidus* L., in which the pollen is ninety per cent. imperfect and the chromosome equipment pentaploid. It is in a variety of this species probably of hybrid origin that Dr. Longley has found the largest number of chromosomes yet exemplified by the genus Rubus, namely, fifty-six or the octoploid equipment.

It would be possible to continue almost indefinitely in the case of Rubus, to point out the highly significant parallelism and agreement of the systematic, genetical and morphocytological evidence as to the hybrid origin of many of the species of the genus. This striking agreement can not be neglected in forming any hypothesis of the origin of species. Nothing can be clearer in the genus under discussion than that polyploidy has arisen in connection with hybridization in nature and that this polyploidy is both of the artioploid and the perissoploid type. In Rubus, accordingly, there is convincing evidence both of the origin of species by crossing and equally of the development of polyploidy as an accompaniment of heterozygosis. If this be admitted, and it is not easy to see how the force of so many harmonizing different lines of evidence can be denied, the same explanation with a high degree of probability can be asserted of the origin of species and of polyploidy in Rosa and Crataegus, on the basis of the cytological investigations of Täckholm and Longley, concerning which we have as yet no genetical evidence. On account of the greater age which must be reached before the gonads are developed, these genera are not well suited for genetical investigation, within reasonable limits of time.

This situation in the case of Rubus is further interesting because of the light which it obviously throws on the conditions in such forms as *Oenothera lamarckiana* and other species of that genus, which in the past two decades have supplied abundant material for the investiga-

tion of the process of mutation, so called. As is well known, *O. lamarckiana* and other species of the genus throw in cultures, types, which were first designated by DeVries, and others, Gigas, Simigigas and Nanella types. Lidforss was successful in finding similar offshoots from known hybrids of Rubus, a situation obviously of the greatest importance in connection with the interpretation of the so-called process of mutation. The various species of Oenothera show the same morphological characteristics as do hybrid species of Rubi, namely polyploidy, irregular meiotic mitosis, polyspory, polycary, lagging chromosomes and chromosomes eliminated into the protoplasm at the time of formation of the daughter nuclei. All these abnormalities belong together and all point towards hybridism as the fruitful cause of the origin of new species. In Rubus the evidence is abundant, many sided and convincing, and this genus will accordingly serve convincingly to illustrate for the present the origin of new species in connection with crossing and resulting polyploidy. There can be little doubt that Datura and Drosophila will be brought under the same rubric in due time. It is interesting to note that Tower in his work on hybrid potato-beetles found that when the members of the uniform hybrid race were segregated they immediately began to "mutate" and to give rise to apparently new species.

Time does not permit me to follow further this interesting theme, but I venture to urge in conclusion that it is important that zoologists and botanists make greater effort to view the process of the origin of species on the basis of the coordinated, comparative and experimental study of the species of large genera rather than on that of the intensive genetical investigation of such isolated and interesting freaks as *Oenothera lamarckiana, Datura Stramonium* and *Drosophila melanogaster.* The purely experimental method appears to have become almost an obsession in biology during the past two decades, and geneticists, in the language of Professor Wilson, have got too far away from their morphological

base. It may be safely predicted that when this many sided and integral study of species becomes general we shall have much clearer and more logical views than at present. In this connection the great importance of the epochal discoveries of Rosenberg on the cytology of hybrids will be realized.

SOME REMARKS ON SPECIES AND CHROMOSOMES

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THE aim of taxonomy, taken in its higher sense, is to describe the forms (species) of the animal and vegetable kingdoms as completely as possible, with the object of helping to explain their relationship and phylogeny, or, in other words, to try to understand their origin and evolution.

Any help to a fuller description of these forms I therefore consider an advance, and I agree with Dr. R. R. Gates that the stating of the number of chromosomes of a form (species) is one more element in its description—and an important one. But I must maintain that the chromosome number does not make the description complete, as there are many other elements of description which are also important.

When Linnaeus described his species, he was satisfied with one or two brief statements of morphological characters. Later we employed much fuller descriptions, and we may eventually take ecological and physiological characters also into consideration, as the microbiologists already do. Some time ago anatomical characters were in vogue, and cytology may be considered as a modern phase thereof, especially the counting of the number of chromosomes for each species. To take a rather wider perspective, the pH-behavior of a form is another modern phase of study which of course also has its importance in a full description of the forms in question.

Now these different features are all merely of a descriptive character, as is also the affirmation of poly-

ploidy in a genus, a discovery which has considerably increased our knowledge of the supposed relationship within certain genera and families. But in being able to understand the origin of new forms (species) and, furthermore, in being able to produce such new forms, we have not advanced very far.

No doubt hybridization plays an important part in the production of new species, and the cytological behavior in hybrids has shown us how the chromosomes arrange themselves. Sometimes we get a stable arrangement, and then the hybrid may be considered a new form (species). Sometimes the arrangement is unstable, and the hybrid splits up. Hybridization does not, however, according to my opinion, help us beyond a certain point, and seems to be most effective in the so-called polymorphous genera. Forms (species) seem to arise in other ways still; we may call them mutations, which only means that we do not know anything about their origin.

What we want to find out—and we have up to the present not many hints to show us the way—are the causes which produce new forms, and still more, we hope in the future to be able to produce the new forms ourselves by inducing changes in the present ones. These changes may be changes in the number of chromosomes, showing themselves also in the alteration of other characters, or it may be changes which, without altering the chromosome number, are visible as new morphological or physiological characters of the form. Until we have compassed this end, we have not reached our ultimate goal—namely, a full knowledge of the origin of species.

ANIMAL PARTHENOGENESIS IN RELATION TO CHROMOSOMES AND SPECIES

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THE subject of this contribution relates to a small facet only of the subject under discussion at this gathering.

It is a small facet because the data at our disposal are meager, but, nevertheless, as no one yet has referred to it in this symposium there is justification for inviting your brief attention to it. If its discussion appears somewhat too speculative it may be asked—surely speculation is one of the *raisons d'être* of these intersectional meetings.

Lotsy, as is well known, champions the theory that hybridization between species gives rise to new species, but it is perhaps not so well known that hybridization, according to Ernst, may give rise to parthenogenesis. This idea naturally follows: Is there any connection between the emergence, through hybridization, of new forms and the emergence, through hybridization, of a new method of propagation of these novelties? Stated in cytological language, have the cytoplasmic and chromosomal constitution of the oocytes of a new hybrid form or the reactions between the two complexes entering into the composition of these oocytes endowed these reproductive cells with the faculty of segmenting and developing without the stimulus of fertilization?

So far as is known among animals, in a group in which thelytokous parthenogenesis is of long standing, as for instance among saw-flies (Tenthredinidae), there is maintained a stereotypy of form. But if we are dealing with a newly produced hybrid which is parthenogenetic we must not expect it, by its very nature, to be parthenogenetically capable of producing offspring diverse both morphologically and sexually; that is unless the oocytes of the F_1 generation hybrid be in such an unstable state chromosomally, and possibly cytoplasmically, that the "simpler" and "short-cut" asexual method of reproduction, in which the complications from union with the male gamete are dispensed with, still does not prevent anomalous meiotic behavior which finds expression in novel forms. Hybridization has even been stated to be a cause of parthenogenesis and such would postulate, as Winkler contends (1), that breeding experiments and

cytological work had demonstrated both the parent species to be parthenogenetic. Considering the exacting nature of such experimentation it is not surprising that a satisfactory demonstration of hybridization as a cause of parthenogenesis does not exist either among plants or animals, though the following isolated zoological example is a close approach. From the botanical evidence of to-day the idea that hybridization is an occasion, but not a cause of the appearance of parthenogenesis, is acceptable, the species crossing, as it were, releasing a latent or slight tendency to parthenogenesis in one or both parents as well as enabling it to develop more fully and to be exploited more freely. However, whatever be true in either or both these views, it makes no difference to the discussion on the rôle of parthenogenesis in the segregation of new and diverse forms, or the repetition, maintenance and fixation of new stereotyped forms, from parthenogenetic F_1 hybrids. So far as I am aware the following results of Harrison[1] (2) afford the only zoological evidence of this thesis comparable to that obtained from the botanical side.

By crossing the two species of moths, *Tephrosia bistortata* and *T. crepuscularia* F_1 hybrids of intermediate character were obtained and one of the females laid parthenogenetically. The parthenogenetically laid eggs yielded an F_2 generation of one female of a more or less nondescript character and two males, one of which was as dark as any specimen reared from normal crossings, while the second was a new form, pale and peppered in appearance. None, however, was comparable with the normal F_2 generation obtained sexually. Beyond this the experiment did not go. Other data relating to the experiment are as follows. The proportion of F_1 hybrids

[1] These results were obtained as a by-product in other genetical experiments, and for other data cited in this contribution and not published in his paper I am indebted to my friend and colleague, Dr. J. W. Heslop Harrison. For the views expressed on these data the present writer, of course, is alone responsible.

which actually laid parthenogenetically was small. Further, compared with conditions in the parents, the number of parthenogenetic eggs laid was fewer and, moreover, the proportion of eggs which hatched was less. Again, the larvae were not easy to rear and their mortality was heavy. Also, there was evidence of anomalous chromosome behavior in the F_1 hybrids. Lastly, repeated and prolonged experiment has not revealed any parthenogenesis in either of the parent species.

We may now discuss the significance of such results. Firstly, whilst it seems possible that parthenogenesis was not inherited from either of the parent species we must await further experimental results in lepidoptera and other animals before asserting that hybridization in these moths evoked parthenogenesis *de novo*. But at least it seems reasonable to conclude that the hybridity and the parthenogenesis were correlated. Have we here a case in which the reactions between the parental chromatins in the parthenogenetic egg have been so violent as to cause parthenogenetic development or, at least, to stimulate anew any inherent latent capacity for parthenogenesis? In view of the well-known results obtained by simple physical and chemical agencies used in artificial parthenogenesis we can not dismiss the possibilities consequent upon the more intimate and vital reactions between different chromatins. That the latter has occurred in this experiment seems evident from the anomalous chromosome behavior. Secondly, the parthenogenetic hybrid, though intermediate compared with the parent species, did not breed a stereotyped race but, by segregation of characters, new forms arose which were diverse in appearance and in sex. By further breeding from this F_2 generation, whether by sexual or parthenogenetic means, new sexual or / and parthenogenetic races are theoretically possible. As the facts stand, however, we have in this case a unique zoological example of hybridity operating by parthenogenesis to produce new forms; that is, assuming the chromosomes

to be the physical basis of heredity, the reactions between the two specific parental chromosomes have given expression to a different mode of inheritance, *viz.*, the parthenogenetic by means of which new phenotypical and genotypical forms have been propagated.

Apparently the only other zoological work which illustrates correlation between parthenogenesis, chromosomal changes and character changes is that of Artom (3). This worker found that the Cagliari race of the brine shrimp *Artemia salina* was bisexual and had a diploid chromosome number of 42 while the Capodistria race was parthenogenetic with a tetraploid chromosome number of 84. But, in addition, the parthenogenetic race showed a somatic difference—the epithelial cells of the mid-gut (endodermal) were larger in size and fewer in number. As is generally admitted for animals, species now parthenogenetic have evolved from a former bisexual condition and such appears to be the case in *Artemia salina.* Hence hybridization and its sequelae of cytological disturbances are ruled out. Rather we are dealing with a case in which the tetraploid nucleus of the parthenogenetic form, in its chromosome content —not in its quality as in the hybrid oocyte—has some relation to the character of the soma. In other words whatever the mode of origin of the tetraploid condition and whatever the causal relation, if any, between tetraploidy and parthenogenesis, we see here a case in which parthenogenesis is utilized as a mode of maintaining a race possessing a form which differs genotypically from the form of the bisexual and parent race.

SUMMARY

(1) Zoological evidence, admittedly meager in amount, concerning the correlation of parthenogenesis with the emergence and propagation of new forms, is presented from the work of Harrison on hybridization of the moths *Tephrosia bistortata* and *T. crepuscularia* and Artom on tetraploidy in the brine shrimp *Artemia salina.*

(2) Harrison's results which show that crossing between the two species has resulted in the production of F_1 hybrids intermediate in character and parthenogenetic in sex which, in turn, have produced a small F_2 generation of new types of both sexes by character segregation, are regarded as illustrating that among animals it is possible that hybridity and correlated parthenogenesis may be responsible for the emergence of new forms.

(3) Artom's results are interpreted as illustrating that parthenogenesis has been closely correlated with the emergence, and has been responsible for the maintenance of the more recently derived tetraploid and genotypically different Capodistria race of *Artemia salina.*

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(1) Winkler, H. Verbreitung und Ursache der Parthenogenesis in Pflanzen und Tierreich, p. 141 *et seq.* Jena, 1920.

(2) Harrison, J. W. Heslop. "The Inheritance of Melanism in the Genus *Tephrosia* (*Ectropis*) with Some Considerations of the Inconstancy of Unit Characters under Crossing." *Jour. of Genetics,* Vol. X. No. 1, July, 1920.

(3) Artom, C. Le basi citologische di una nuova sistematica del genere Artemia. *Archiv für Zellforschung* 9. Band, 1. Heft, 1912.

APPENDIX

Since writing the above, the writer has just obtained an interesting corroborative result from material kindly supplied by Dr. Harrison, particulars of which are as follows. From a certain experiment in 1923, related in some detail in *Nature,* August 18, 1923,[2] Dr. Harrison obtained in February, 1924, a melanic male of *Tephrosia bistortata,* melanism being recessive. This male was crossed with a type (white) *T. crepuscularia* female in February, 1924, and the resulting F_1 hybrid generation of May-June, 1924, were of both sexes and light like the female parent. One of these light colored hybrid females laid 200 to 300 unfertilized eggs though only 6 larvae emerged. These larvae, newly hatched, were handed to

[2] Garrett, F. C., and J. W. H. Harrison. Melanism in Lepidoptera and its possible induction. *Nature,* Aug. 18, 1923, p. 241.

me and reared on knotgrass (*Polygonum aviculare*) but only two reached the pupal stage; the others, for some unaccountable reason, died. The two pupae wintered from July, 1924, and in January, 1925, precise date uncertain, one of the moths emerged but, unfortunately for further experiment, was dead when discovered. It was a melanic female. The second pupa on examination was found to be dead but it had developed sufficiently far to show that it was a melanic male. The experiment may be set out thus:

Melanic *T. bistortata* ♀ ; ww ×
Normal white *T. crepuscularia* ♀ ; WW
|
White hybrid ♀ ; Ww; parthenogenetically has given rise to
|
Two pure recessive melanics; 1 ♂ and 1 ♀ ; ww.

It is curious and interesting that the two specimens which survived to an identifiable stage were melanic recessives when the chance of such forms appearing was only one in four even under ordinary Mendelian conditions.

Dr. Harrison also informs me that despite repeated attempts since the specimens mentioned in the above contribution were initiated—six years ago—he has never again obtained parthenogenetically laid eggs until May, 1924.

THE PHYSIOLOGICAL SIGNIFICANCE OF CONJUGATION AND ENDOMIXIS IN THE INFUSORIA[1]

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THE first serious attempt to give a comprehensive survey of the Protozoa is afforded by the studies of Otto F. Müller made during the latter part of the eighteenth century, though it is true that Dr. John Hill in his "History of Animals," published in 1752, stated that "their want of magnitude does not exclude them from their rank among animated beings . . . I have arranged them into a regular method, and given them denominations"—and one denomination he coined was Paramecium. But Müller, profiting by the microscopical studies in the interim, as well as by the establishment of the Linnaean system of classification, produced in his Animalcula Infusoria the actual base line for future investigations. Our present interest, however, in Müller's work is due to the fact that he rightly interpreted pairs of Infusoria as undergoing a sexual process and not as dividing longitudinally. The latter, strange to say, was the view of his successors down to 1858, when Balbiani published his observations on the existence of sexual reproduction in the Infusoria.

Balbiani thought that the nucleus and micronucleus became transformed into sexual organs—the macronucleus producing eggs, and the micronucleus, sperm—and he thereby obscured a minimum of fact with a plethora of imagination. Nevertheless, with Balbiani's work as the point of definite departure, the problem at the hands of Bütschli and Engelmann in 1876 was placed well upon the road toward its present-day development. These authors established the dimorphic nature of the nuclear

[1] Lecture delivered at the Woods Hole Marine Biological Laboratory, July 18, 1924.

apparatus of the Infusoria and the formation of a new macronucleus from the micronucleus during conjugation, though it remained for a series of workers during the eighties of the last century—notable among them being Balbiani, Maupas and Richard Hertwig—to observe the important cytological features of conjugation, including synkaryon formation, in the Protozoa.

But both Bütschli and Engelmann did more than supply cytological data: they offered the first concrete suggestions in regard to the significance of fertilization. Bütschli believed it to be a process of rejuvenation, and Engelmann one of reorganization: a difference in details, but an agreement in fundamentals. Both thought that continued reproduction by division tends to slow down through loss of vitality, and that fertilization restores the pristine vigor of the cells. And thus the so-called "dynamic" interpretation of fertilization phenomena in the Protozoa arose.

These pioneer studies of fertilization in the Protozoa were made during and, it is probably fair to say, under the stimulus of the classic period of cytological advance. Bütschli and Auerbach, Oscar Hertwig and Fol, Flemming, van Beneden and Boveri were establishing the essential features of mitosis, maturation and fertilization in metazoan eggs which gradually led to the full realization of the genetic continuity of the germ nuclei foreshadowed a generation before. And this, in turn, formed the indispensable prelude to the elucidation of the chromosome mechanism which cytologists have supplied to geneticists as the physical basis of heredity.

Thus was added content to the view, emphasized two centuries before by Harvey and modernized by Oscar Hertwig and Minot, that fertilization supplies a stimulus to egg development, and to the equally ancient and probably more important one that fertilization affords the basis of biparental inheritance in all sexually reproducing organisms.

The answers to several fundamental physiological problems have been sought in the study of the Infusoria,

which may be stated in the form of two general questions:

(1) Do these organisms possess the power to reproduce indefinitely by division, under favorable environmental conditions, without recourse to conjugation or to endomixis?

(2) In cultures which are exhibiting lowered vitality, does either conjugation or endomixis restore the normal metabolic condition and "rejuvenate" the organisms?

We shall consider these and attempt to present definite answers, but it is unnecessary to state that since there are always at least two sides to every question, impartial as the reviewer wishes to be, the personal equation must perforce find expression.

The first consistent investigation of the power of the Infusoria to reproduce indefinitely without recourse to conjugation is afforded by the elaborate studies of Maupas in which he presented a wealth of data and a series of interpretations that for more than a decade were taken at their face value and believed to prove that the life history runs in cycles. In brief, a long period of multiplication by cell division is succeeded by an epidemic of conjugation, which inaugurates a new cycle, and is comparable in its physiological aspect with the period of sexual maturity in the metazoa. If conjugation does not occur, the race rapidly degenerates and dies, and accordingly conjugation counteracts the tendency to senile degeneration and causes rejuvenescence.

Now to-day we are asking whether Maupas's data show what he says, and whether he says what he means! This is not merely of historic interest because his hundreds of pages filled with observational and experimental details have had great weight.[2]

Maupas definitely says, "My cultures have experimentally shown that these Protozoa do not escape the general law of senescence. They exhaust themselves and become old by the exercise of their functions, or, in other words,

[2] Calkins, "What did Maupas mean," AMER. NAT., 57, 1923.

because of a necessity inherent in their structure and constitution." This, as Calkins points out, is exceedingly explicit and is the burden of Maupas's entire experimental study.

But Maupas states that his records indicate that "the power of reproduction follows no such diminishing and parallel course. It is maintained almost intact even a long time after the other functions and the entire organism are shown to be greatly reduced by senile degeneration . . . the Infusoria, although feeble and much reduced in size, continue to divide with almost the same rapidity as at the beginning." In other words, Maupas apparently believed that progressive physiological and morphological degeneration is compatible with a normal division rate. As Calkins's analysis has shown, Maupas's own data do not indicate the biologically untenable conclusion that division rate, which subsequent investigation has amply demonstrated is a most subtle index of metabolism, is unaffected by degenerative changes.

So, although we can at least accept it as a fact that Maupas found progressive degeneration as cell division proceeded, we must reject his statement that the division rate was unaffected. His studies indicate cyclical change, both structural and physiological, during interconjugation periods. And thus the so-called cycle definitely entered the literature on the Infusoria.

Without minimizing the importance of the pioneer work of Maupas, it is nevertheless true that his method of culture was inadequate to the problem, and the great advance which paved the way for all more recent work was the adoption of the daily isolation method by Calkins when he took up the problem after Maupas's results had been accepted for over a decade. This method rendered possible careful control of the culture medium from day to day, accurate records of the rate of division and the positive elimination of conjugation. In brief, it afforded for study precise pedigree cultures of Infusoria.[3]

[3] Calkins, "Studies on the life history of Protozoa. I. The life cycle of Paramecium caudatum," *Arch. f. Entw-mech.*, 15, 1902.

Calkins's investigations on the question of the cyclical nature of the life history of Infusoria began in 1901 with Paramecium caudatum and have been continued on various species, culminating in an extensive analysis of the life history of Uroleptus mobilis which is still in progress. His answer to the question under consideration is that the "physiological processes of metabolism are not capable of unlimited activity . . . there is a progressive weakening of metabolic vigor from an optimum shortly after conjugation." "Such weakened protoplasm, if not allowed to conjugate, inevitably dies, as does the somatic protoplasm of metazoa." "Natural death from old age . . . is actually inherent in protoplasm as little differentiated as in these single-celled animals."[4]

But other workers, following the lead and using, in general, the technique of Calkins, have reached other results and conclusions. Enriques has bred cultures of several Infusorians, notably Glaucoma pyriformis, for nearly 3,000 generations, without signs of lowered vitality. At New Haven, Paramecium aurelia has been carried for some fifteen years and more than 10,000 generations, and, among others, Spathidium spathula for over a year, during which 1,000 generations were attained. Conjugation has not occurred in the direct line of any of the cultures. Similar results have been attained by Baitsell on Pleurotricha lanceolata and Oxytricha fallax, by Mast on Didinium nasutum and by Dawson on Oxytricha hymenostoma.

It is highly significant that the longevity of Infusoria —the so-called life cycle—has been gradually though consistently prolonged year by year as pedigree culture technique has been perfected. Even such relatively ubiquitous forms as Paramecium aurelia and caudatum, which are able to survive temporarily under very diverse conditions, gradually succumb to prolonged subjection to media which at first seem entirely suitable. Ample evi-

[4] Calkins, "Uroleptus mobilis, II, Renewal of vitality through conjugation," *J. Exp. Zool.*, 29, 1919.

dence of this is afforded by the history, during more than threescore years, of the study of Paramecia cultures.

Quite a similar history has been briefly recapitulated at New Haven in the study of Spathidium spathula during the past four years. The majority of the cultures showed what heretofore would have been interpreted as inherent "cycles" which culminated in death unless conjugation was permitted, but the drift toward longer life of certain cultures as the experiments progressed, and finally the attainment, as just mentioned, of more than a thousand generations with every indication of the potentiality to continue to divide indefinitely, can, we believe, be reasonably interpreted in but one way.[5]

The only species extensively bred which still proves refractory is Uroleptus mobilis. The extreme rarity of this species may reasonably, we believe, be attributed to peculiar conditions of life demanded, and its cycle to the fact that some crucial factors of the milieu have thus far not been supplied. If this is not the proper interpretation of the cyclical character of the Uroleptus life history, then this form certainly suffers a limitation which is not shared by the other species investigated.

To one question, then, we believe an unequivocal answer has been given—the Infusoria in general can, under favorable environmental conditions, reproduce indefinitely by division without recourse to conjugation. The cycle is a phantom which is slowly but surely receding toward the vanishing point, as the special requirements of particular organisms are being established.

From the study of various species of hypotrichous Infusoria as well as from the long continued Paramecium aurelia culture at New Haven, it was found that minor periodic fluctuations of the division rate occur, from which recovery is autonomous. These fluctuations were termed rhythms and contrasted with the so-called

[5] Woodruff, "The present status of the long continued pedigree culture of Paramecium aurelia at Yale University," Proc. Nat. Acad. Sci., 7, 1921.
Woodruff and Moore, "On the longevity of Spathidium spathula without endomixis or conjugation," Proc. Nat. Acad. Sci., 10, 1924.

"cycle," which comprises a varying number of rhythms and, according to Calkins, ends in the death of the race if conjugation or artificial stimulation is not resorted to.[6]

An intensive study of rhythms was made in Paramecium aurelia which showed that subjection of the culture to the most constant environmental conditions failed to eliminate the rhythms, but, instead, they appeared still more pronounced. It was also found from a study of the temperature coefficient of the rate of division that this is influenced by temperature at a velocity similar to that for a chemical reaction, except when the rhythms interfere. Thus it became apparent that there are inherent rhythmical changes in the phenomena of the cell which produce slight fluctuations in the rate of division.[7]

Daily cytological study of pedigree Paramecium aurelia and caudatum showed that the rhythms in these species are coincident with a nuclear reorganization process which was termed endomixis. It will be recalled that an essential difference between endomixis and conjugation is the absence of the third micronuclear division, which in conjugation forms the gametic nuclei, and, of necessity, the non-formation of a synkaryon.[8]

This process of endomixis—or, if you will, parthenogenesis, though we believe the non-committal term preferable at the present stage of our knowledge—has also been found in one other species of the genus Paramecium, P. polycaryum; Calkins has found it in Didinium nasutum and Uroleptus mobilis, and Moore in Spathidium spathula. Fermor described an internal reorganization process during encystment in Stylonychia pustulata which, however, involves a nuclear fusion and is, therefore, not endomixis but autogamy.[9]

[6] Woodruff, "An experimental study on the life history of hypotrichous Infusoria." *J. Exper. Zool.*, 2, 1905.

[7] Woodruff, "The influence of general environmental conditions on the periodicity of endomixis in Paramecium aurelia," *Biol. Bull.*, 33, 1917.

Woodruff and Baitsell, "The temperature coefficient of the rate of reproduction of Paramecium aurelia," *Amer. J. Physiol.*, 29, 1911.

[8] Woodruff and Erdmann, "A normal periodic reorganization process without cell fusion in Paramecium," *J. Exp. Zool.*, 17, 1914.

[9] Fermor, "Die Bedeutung der Encystierung bei Stylonychia pustulata," *Zool. Anz.*, 42, 1913.

The discovery of endomixis in Paramecium aurelia and caudatum coincident with rhythms which are due to intrinsic and not external factors naturally led to the interpretation of endomixis as the phenomenon involved—a rhythm thereupon being regarded as the outward physiological expression of the underlying cytological phenomena of endomixis. Furthermore, it was shown in Paramecium aurelia and caudatum that cessation of endomixis is soon followed by the death of the culture. This affords the best possible proof that a periodic occurrence of endomixis is indispensable for the continued life of these species.

So far as Paramecium aurelia and caudatum are concerned, the rhythm may perhaps be considered as an expression of a sort of temporary "senescence" for which is provided an internal, automatic antidote in the form of endomixis. But I would emphasize that this is a case of trying to force new wine into old bottles in order to save an idea. One certainly must grant that the original idea of senescence in Infusoria is far removed from this subtle, automatically arrested type which rhythms may indicate. To shrink the cycle to the rhythm is to annihilate the cycle.

But we are not interested in an academic discussion of terms—the fact remains that still more recent work has shown that in other species of Infusoria endomixis does not occur during the free life of the cell, though rhythms, as originally defined, are present. This is the case in Spathidium spathula—endomixis occurring, as Moore has shown, during encystment; and in Paramecium calkinsi, in which endomixis, as demonstrated by Spencer, does not take place at all. In other words, endomixis occurs at the low point of the rhythms in Paramecium aurelia and caudatum, and though, from the very nature of the process, it accentuates the drop in the division rate, nevertheless it is not the underlying factor of rhythms after all—for rhythms exist *without* endomixis in other species.

Such being the case, the whole problem takes on another aspect: we have two phenomena to consider—rhythms and endomixis. For the moment, however, we are seeking a definite answer to the specific question whether Infusoria can reproduce indefinitely by division without endomixis. It is clear that Paramecium aurelia and caudatum can live indefinitely without conjugation, but that suppression of endomixis inevitably is followed by death, and accordingly endomixis is indispensable in the life history of these species. But Enriques states that endomixis did not occur in his long-continued Glaucoma culture, and at New Haven it has been shown that Paramecium calkinsi, which does not undergo endomixis, apparently can reproduce indefinitely by division, and that Spathidium spathula, which undergoes endomixis solely while encysted, can reproduce indefinitely by division without encystment and, therefore, without endomixis.[10]

Accordingly, although the suppression of the endomictic process while in progress in the free life of Paramecium aurelia and caudatum is fatal, the undoubted fact that in other species of Infusoria it may be eliminated by preventing its onset (*e.g.*, by preventing encystment) and in still other species it apparently does not occur at all, forces us to the definite conclusion that endomixis is not a fundamental, indispensable factor in the life history of the Infusoria—that they can reproduce indefinitely by division under favorable environmental conditions.

In brief, the time-honored question whether the Infusoria degenerate and die when conjugation is prevented has, we believe, been answered unequivocally in the negative, and likewise, the very new question whether Infuso-

[10] Enriques, "Duemila cinquecento generazioni in un infusorio, senza coniugazione nè partenogenesi, nè depressioni." R. Acad. Sci. Bologna, 1916.

Moore, "Endomixis and encystment in Spathidium spathula," *J. Exp. Zool.*, 39, 1924.

Spencer, "Studies on Paramecium calkinsi," *J. Morph. and Physiol.*, 39, 1924.

ria in general degenerate and die when endomixis does not take place has also been answered unequivocally in the negative. Therefore, not only is synkaryon formation unnecessary, but a periodic reconstitution of the nuclear apparatus from the micronucleus is unnecessary. All the evidence cumulatively points to the conclusion that the zygotic nucleus constituted during syngamy and the nucleus constituted during endomixis in the Infusoria are adequate for indefinite division of the cell under favorable environmental conditions.

Having eliminated, to our satisfaction at least, amphimixis, endomixis and the cycle we still have the rhythm. To what are rhythms due? Defined from a study of the life history of hypotrichous Infusoria in 1905 as "a minor periodic rise and fall of the fission rate, due to some unknown factor in cell metabolism, from which recovery is autonomous" they have since then been in evidence in every culture of Infusoria whose division rate has been published. Endomixis in Paramecium aurelia and caudatum was believed to offer the solution, but it is evidently not the cause of the rhythms—though it accentuates them in these species—since our belief that endomixis would be found during the rhythms of every species has not proved to be correct. We are forced, therefore, to fall back for the present on our general statement of twenty years' standing—"due to some unknown factor in cell metabolism."

But there is one possibility which, remote as it is, must not go unmentioned. Rhythms in Infusoria synchronous with endomixis may not be the same phenomenon as rhythms in forms which do not undergo an endomictic process. Although the term was originally applied to fluctuations in the fission rate of species which subsequent work has shown do not have endomixis, and then applied to fluctuations in the rate of Paramecium aurelia which later work has shown does undergo endomixis, it is a fact that the most extensive attempts to eliminate these fluctuations by constant environmental conditions were made

on Paramecium aurelia before endomixis was known. An equally intensive study of a form without endomixis might show that the rhythms can be eliminated, although Spencer has already carried on a series of cultures of Paramecium calkinsi for this particular purpose which makes it almost certain that the rhythms are inherent.

We have considered the question whether conjugation is a necessary factor in the life history of the Infusoria and have answered it in the negative. But, obviously, because conjugation is not necessary under favorable environmental conditions, it does not follow that it is not necessary under other conditions—that it does not have a dynamic function which is brought into play when the organism meets various vicissitudes of its environment.

In opposition to the prevailing view based chiefly on the work of Bütschli, Maupas insisted that conjugation does not increase the rate of reproduction: it merely wards off senile degeneration when it occurs before this is far advanced. Richard Hertwig from very limited experimental evidence reached similar results, except that he found after conjugation the division rate was *lower* than before—and so he interpreted the rejuvenating effect of conjugation as due to a repression of division energy.

However, the work which is significant to-day begins with Calkins's studies carried out twenty years ago on Paramecium caudatum. In degenerating pedigree cultures of this organism, he found that so-called rejuvenescence occurs after exogamous or endogamous conjugation, but only in a small percentage of cases, for only a few unions result in continued life of the exconjugants. These failures, he thought, indicate that only certain combinations are capable of yielding fertile results, especially in view of the fact that he obtained a high percentage of viable conjugants from wild Paramecia. From further work on the same organism he concluded that conjugation between two cells results in the complete reinvigoration of all activities, both physiological and germinal.

So the problem stood when Jennings in 1913 published his extensive study on the effects of conjugation in Paramecium, in which he marshalled the data from Maupas and Hertwig to support his own brilliantly attained result, from experimental and biometrical analysis, that "the average rate of reproduction is less after conjugation than before." That is, when he took, for example, two sets of animals of the same stock and history, both ready to conjugate; permitted one set to conjugate and prevented the other, he found that those which had conjugated divided thereafter on the average less rapidly than the others. In most cases the rate of fission was very considerably greater in those that had not conjugated, and in no cases did the conjugants have a higher rate of fission. Furthermore, as a rule mortality was much higher, under the same conditions, among animals that had conjugated than among those which had been prevented from conjugating—regardless of whether the animals were unrelated or were members of the same race. So Jennings naturally asked: "Is it not time that the statements or implications that in the Infusoria conjugation results in increased reproduction should disappear from the literature of science?" And four years later Mast's studies on Didinium nasutum led him to agree that conjugation has no appreciable effect on the death rate, fission rate or variation in the fission rate.[11]

In 1917 Calkins resumed his study of the problem, employing a pedigree culture of the hypotrich Uroleptus mobilis. Carrying more than thirty series from exconjugants, he found that there was a progressive weakening of metabolic vigor from an optimum shortly after conjugation. This progressive weakening he attributes, as we have stated, to inherent senescence, while we would attribute it, as we have our earlier results with Spathidium, to extrinsic causes. However the condition arose, the significant fact for the moment is that the animals

[11] Jennings, "The effect of conjugation in Paramecium," *J. Exp. Zool.*, 14, 1913; "Life and Death, Heredity and Evolution in Unicellular Organisms," Boston, 1920.

were transformed from a condition of metabolic weakness to a condition of optimum metabolic vigor by the process of conjugation. Animals which were not allowed to conjugate, under the conditions of the experiment, died.

Now since Jennings showed on a large scale in his studies "that conjugation brings about great variation in vitality and reproductive power" in Paramecium, it is perfectly reasonable to regard single examples of increased fission rate following conjugation as one of the rare extreme variants. But Uroleptus shows almost without exception stimulation following conjugation, so that this explanation is no longer tenable; and Calkins in these experiments has certainly supplied the first apparently unassailable evidence that conjugation is followed by renewal of vitality as indicated by increased reproductive power.

Before 1920 our studies were directed to the question: What happens when conjugation is prevented? But then we turned to the question: What happens when conjugation occurs? Spathidium spathula was selected for the work after a long search for favorable material because it affords the several features which are essential for the successful attainment of the desired data: sufficient size to render practical the daily isolation of large numbers of animals; the non-occurrence of endomixis in free living stages; and, most important of all, the frequent onset of epidemics of conjugation between pedigree animals so that non-conjugant and conjugant lines can be secured and compared at crucial periods. Conjugation could be obtained almost at will by allowing a few animals to remain on a culture slide for a day or two without an adequate supply of Colpidia, which is the staple diet of Spathidium. Incidentally, it may be mentioned that Moody's experience with Spathidium was quite different in that no evidence of ability to conjugate was evident, apparently because it was an amicronucleate race.[12]

[12] Woodruff and Spencer, "Studies on Spathidium spathula, I, The structure and behavior of Spathidium," *J. Exp. Zool.*, 35, 1922; "II, The significance of conjugation," *J. Exp. Zool.*, 39, 1924.

All the animals used in our studies were descendants from a single "wild" animal collected at New Haven. Standard beef extract was employed as the basic culture medium, and to this was added, as used, Colpidia raised in flasks of hay infusion. All the cultures directly compared were bred simultaneously under identical culture conditions.

The general method of conducting the pedigree lines may be briefly stated. From the original animal four lines were carried as the stem culture. Animals left over at the daily isolation were kept as stock, or allowed to conjugate or encyst. From time to time pairs of conjugants were isolated and their progeny established as "new" pedigree groups of cultures from the stem culture or its derivatives. Usually these groups comprised the progeny of two pairs of conjugants, though in a few cases the progeny of other pairs were also carried. In every instance the culture derived from a single exconjugant comprised four lines of animals and the average daily division rate of these four lines, again averaged for five-day periods, forms the basic data. At certain periods nearly 200 isolations were made daily, so that the investigation involved the manipulation of upward of 100,000 pedigree animals.

Passing over the question of the existence of a normal, inherent cycle in the life history of Spathidium, which we have already answered in the *negative* (cf. p. 230), we may summarize the data in regard to the effects of conjugation in cultures exhibiting a lowering of vitality under laboratory conditions.

A total of 118 exconjugants contributed to the experimental results. Of these, 18 died without dividing—a positive result—death immediately followed conjugation. However, the proportion of deaths is so small that it is negligible from the standpoint of this study. It is lower than the exigencies involved in such experimental work would naturally supply when the isolations and manipulations necessary are taken into account. Indeed,

the high viability of exconjugants is remarkable in comparison with that obtained by most investigators studying similar problems. In addition to the cases just mentioned, 6 other exconjugants need not be considered since they were lost by accident. Thus there remain 94 exconjugants which established cultures affording cogent data, of which 79 ran synchronously with their parent cultures for three or more five-day periods, and so supply definite evidence bearing on the question: Do the progeny of exconjugants characteristically exhibit a significantly different (higher or lower) rate of reproduction from that of their parent culture which has not so recently conjugated?

A study of the initial fifteen days of each of the 79 exconjugant cultures shows that 67 divided more rapidly and 12 less rapidly than their respective parents. The 15 other cultures, whose parental cultures died within the fifteen days, all actually divided more rapidly than their respective parents, so that they may with justice be added to the regular 67 plus cultures to give 82 cultures showing an initial increase in the division rate. However, it is immaterial whether the latter cases are included or not, so far as the conclusion which must be drawn is concerned. Obviously, the data overwhelmingly indicate that the rate of reproduction in the great majority of cases is accelerated after conjugation.

But the analysis thus far has involved merely contrasting the division rate of exconjugant and parent cultures on the basis of an increase or decrease of division rate, without considering the amount of difference. Clearly, before a conclusion is secure, it is necessary to take into account the chance variation which is inevitable in any experiment in animal breeding such as the present. The usual biometrical methods of analysis are inapplicable here because in each experiment the individual cultures must be treated separately, and judged in relation to the synchronous division rate of their respective parent cultures and also of their grandparental

cultures (if still surviving), as well as in relation to other cultures.

But the difficulties are more fancied than real, for by any reasonable criterion the number of cases in which the exconjugants exceeded the parent is far and away beyond the limits of any probable error that statistical methods may formulate. We have adopted a difference of five divisions in three periods, or an average of one third of a division per day, as a significant variation. Applying this criterion to the data gives 57 exconjugant cultures exceeding their respective parent cultures; 6 exconjugant cultures below their parent cultures, and 16 which are within this limit of error. A similar analysis in which seven divisions in three periods is taken as the significant variation gives 50 plus exconjugants, 5 minus exconjugants and 24 neutral. But our experience in handling the hundreds of pedigree lines during this experiment has convinced us that seven divisions in three periods is without doubt unreasonably large, and this is obvious when it is realized that this represents a change in the average rate of nearly one half a division per day. Nevertheless, even with this ultraconservative standard, the data give an unequivocal answer: conjugation in the great majority of cases directly induces an immediate acceleration of the reproductive activity.

A further analysis of the data, which need not be made at this time, fairly establishes for this pedigree race of Spathidium that conjugation usually is even more effective in accelerating reproduction when the exconjugants are derived from slowly dividing parents than when they are derived from rapidly dividing parents.

Turning now to the later effects of conjugation, we have 81 cultures which afford available data. Of these, 76 exconjugant cultures exceeded their parental cultures in the total number of divisions attained during their synchronous life, and 5 exconjugant cultures underwent a smaller number of divisions. Obviously this indicates that following conjugation the rate of division is accelerated.

But again, this analysis does not take into consideration the amount of difference in division rate between exconjugant and parental lines, and the difficulties involved here in determining a variation which is significant are still greater than in the comparison, just concluded, for the rates during a definite period of fifteen days. For now we are dealing with differences in duration: *e.g.*, some cultures ran synchronously for three periods and others for more than a dozen periods. Probably we can not do better than to take again the average variation of one third of a division per day as the criterion of a significant variation. Judged from this standard, 60 exconjugant cultures exceeded their parental cultures in division rate during their entire synchronous life, 3 exconjugant cultures were lower in division rate, and 18 cultures were within the limit of error of our standard.

Since conjugation is the sole variable involved in exconjugant and parental cultures, it is evident that conjugation directly induces not only an immediate acceleration of reproduction, but also an acceleration which persists at least as long as the life of the parental culture. And it is significant that the data from the first three periods and also from the entire synchronous life are practically identical, since this shows that the initial acceleration is maintained in essentially equal intensity to the end.

The question now to be considered is whether the progeny of exconjugants characteristically exhibit an increase or decrease in longevity in comparison with their parent culture which has not so recently conjugated. Among the 70 cultures which afford cogent data, 54 exconjugant cultures continued to live and reproduce after the death of their respective parent cultures, 10 exconjugant cultures died before their respective parent cultures, and 6 exconjugant cultures died essentially synchronously with their respective parent cultures. This analysis includes the cases where the exconjugant cultures when dividing rapidly suddenly died out in a day

without preliminary signs, because no criterion exists for positively determining that such deaths were "accidental." But since this fate befell more exconjugant than parent cultures, if these were omitted from the analysis the relative number of exconjugant cultures which survived their parent cultures obviously would be increased.

Thus the data presented provide a very definite answer. In the great majority of cases the exconjugant cultures outlived their parent cultures, and since the advent of conjugation is the sole variable involved, it is apparent that conjugation tends to increase the time duration of life as well as the rate of reproduction in Spathidium. Therefore, necessarily, the number of generations attained by the exconjugant cultures exceeds that of the parent after the origin of the former. In brief, under the conditions of these experiments, conjugation has a survival value in the life history of this organism.

This conclusion naturally leads to a consideration of the effect of conjugation on animals from cultures which are actually on the verge of extinction, in order to determine if conjugation has a direct and immediate survival value.

Four groups of cultures offer definite data. In each instance the progeny of the exconjugant survived while the parent culture died. And this evidence, direct as it is, has been amply corroborated in a very practical way by all the other work on Spathidium which for more than four years has been carried on at New Haven. Cultures about to die have been continued with exconjugants. It is clear, therefore, that conjugation, in the great majority of cases, has a direct survival value.

The relation of conjugation to the continuance of the life of the pedigree race from the parental to the F_5 generation under cultural conditions can be best illustrated by a specific example. From the parental culture at the thirtieth division the F_1 generation was derived; from

the F_1 at the 104th division, the F_2 generation was derived; from the F_2 generation at the 289th division the F_3 was derived; from the F_3 at the 150th division the F_4 was derived; and from the F_4 at the 80th division the F_5 was derived. The latter died at the 300th division.

Thus, from the parental culture to the death of the F_5 generation the series of pedigree cultures extended from November 5, 1920, to November 5, 1921, exactly a year, and the series could, without doubt, have been continued indefinitely by the same procedure. During the year the actual number of "linear" cell divisions from the parental culture through the F_5 generation of this series was 953. Since, under the conditions of the experiment, the parental culture died after 234 divisions on February 11, 1921, it is obvious that the life of the series, by five "conjugations" was prolonged approximately nine months, and exactly 719 divisions. Data may be similarly analyzed for the various generations of other groups, but it is unnecessary to do so here.

In brief, nothing can obscure the fact that under identical culture conditions "new" cultures, derived from conjugating pairs in an "old" culture, consistently survived the parent cultures. This, it appears, is a further definite demonstration of the survival value of conjugation.

The gradual "building up" of the division rate of a series through successive "conjugations" is also readily shown by the division rate of the same series of cultures which we have just been considering. The parental culture started with an initial rate of slightly above four divisions per day which was never again attained throughout its life. The F_1 generation attained but never exceeded the division rate of its parent. The F_2 generation never attained the maximum of its parental and grandparental cultures. The F_3 generation derived when its parent was dividing less than one and one half divisions per day and rapidly approaching extinction, exhibited a restoration of division energy which reached

three and one half divisions per day. However, this is below the initial maximum of the previous generations. The F_4 generation, derived when its parent was dividing approximately twice per day, immediately attained and maintained until discontinued a rate of nearly four divisions per day. This is almost as high as the maximum of the P and F_1 generations, identical with the initial maximum of the F_2 generation, and much higher than that ever reached by the F_3 generation. The F_5 generation, derived when its parent was dividing at an average rate of nearly four times per day, exhibited an initial rate of nearly four and one half divisions daily, the grand maximum for the entire series.

Granting that the series selected as an example is the most striking case the data afford, the fact remains that the other cultures, directly involved in the specific experiments to "build up" the division rate to a maximum equal or better than that ever exhibited before by this pedigree race of Spathidium, consistently show a similar cumulative effect of conjugation. Accordingly, all the data involved demonstrate that it is possible by successive "conjugations" progressively to increase, within limits, the maximum division rate.

Throughout all these experiments the animals employed were descendants from the one "wild" Spathidium isolated to start the stem culture, and therefore each generation (F_1 to F_5) in the series was the product of close inbreeding. As a matter of fact, the two animals which conjugated were in no instance more than eight divisions removed from a common ancestor. Since, as has been shown, conjugation effects an equal, if not greater, acceleration of the reproductive rate during the later experiments, it follows that continued close inbreeding, at least to the F_6 generation, does not significantly alter the typical physiological effect following conjugation at the F_1 generation. But in order to compare exconjugant cultures arising by such close inbreeding with similar cultures arising from the crossing of distantly

related animals, an attempt was made to cross animals which were separated by a large number of generations. The difficulties involved have thus far resulted in only one experiment out of over four hundred being satisfactorily carried through to completion. The animals mated were actually 982 divisions removed from a single common ancestral animal.

This culture and the necessary control cultures, involving 44 animals whose division rate was recorded daily for 25 days, showed no significant difference in rate of reproduction between the progeny of conjugants which were not more than eight divisions (about 48 hours) removed from a common ancestor and the progeny of the cross-conjugants, the members of which were removed 982 divisions (185 days) during which five "conjugations" were interpolated. Although no special weight should be given to this result, so far as it goes it is in accord with the data in regard to inbreeding from our other cultures.

In brief, all the data regarding the effects of conjugation derived from our study of Spathidium indicate a profound physiological stimulation of the metabolic activities of the cell which is expressed in reproduction.

As we know, endomixis is a periodic process coincident with the rhythms during the free life of Paramecium aurelia, P. caudatum and P. polycaryum.[13] In Paramecium calkinsi, endomixis is absent though rhythms occur; in Didinium and Uroleptus, according to Calkins, and in Spathidium, according to Moore, it occurs during encystment. In Paramecium calkinsi it obviously is not a necessary phenomenon, and the same conclusion has been drawn from data on Spathidium. But, when it does occur, has it a significant physiological effect?

In Paramecium aurelia and P. caudatum a higher division rate certainly follows endomixis, since recovery from a rhythm is coincident with endomixis. However, since

[13] Woodruff and Spencer, "Paramecium polycaryum, sp. nov.," Proc. Soc. Exp. Biol. & Med., 20, 1923.

in other Infusoria (*e.g.*, Paramecium calkinsi) recovery from rhythms regularly occurs in the entire absence of endomixis, it is impossible to definitely determine a causal relation between endomixis and recovery from rhythms in Paramecium aurelia and P. caudatum.

In pedigree cultures of Didinium, Calkins found that periodically all the animals encysted for purposes of reorganization, or endomixis, and accordingly he interpreted the process as possessing a survival value for the race. Mast in his studies of the same species did not find periodic encystment and stated it to be improbable that encystment is necessary for the continued existence of Didinium or that it has any appreciable effect on the death rate or fission rate.[14] The data from Uroleptus, however, certainly indicate, as Calkins says, that after encystation, during which endomixis takes place, the animals possess an initial vigor similar to that of an exconjugant. Furthermore, split conjugants, that is, animals separated or cut apart immediately after contact and therefore long before the definitive nuclear changes of conjugation have occurred, undergo an endomictic process which results in rejuvenescence exactly similar to that following conjugation. Apparently the mere contact of two animals initiates the nuclear changes for conjugation, and when conjugation is thwarted the cells meet the emergency by an endomictic process which typically only takes place in the species during encystment.

Weighing all the available facts the conclusion seems justified that when degeneration is in progress endomixis has a rejuvenating effect, and we thus reach the general result that *both* conjugation and endomixis have a similar effect under similar conditions—both meet the emergency of physiological degeneration *induced by environmental conditions* which are not ideal. The fact that Paramecium aurelia and P. caudatum are so widely distributed and are able to exist under such diverse surroundings

[14] Calkins, "Didinium nasutum, I, The life history," *J. Exp. Zool.*, 19, 1915; Mast, "Conjugation and encystment in Didinium nasutum with special reference to their significance," *J. Exp. Zool.*, 23, 1917.

can reasonably be interpreted as due to their having instituted endomixis in the free-living state. And furthermore, it is possible, we believe, to explain in part, at least, the fact that Jennings's remarkably intensive study of conjugation in Paramecium failed to reveal acceleration of cell division: endomixis, which was then unknown, tended to maintain his control lines at a high level. At all events, crucial experiments on the physiological effects of conjugation are only possible in forms in which endomoxis is not a complicating factor.

If the conclusion is secure that both endomixis and conjugation rejuvenate, it is clear that synkaryon formation is not the essential factor, since this does not occur during endomixis. The cytological evidence, which is solely from Paramecium, indicates that in endomixis the first two of the three 'pre-synkaryon' divisions occur, one of which during conjugation is usually interpreted as a reduction division, though recently Dehorne has claimed that it is the third division. Since, however, the nuclear changes in conjugation and endomixis are apparently fundamentally the same except for the gamete-forming division, endomixis in Paramecium offers strong physiological evidence against the interpretation of either of the first two divisions in conjugation as effecting chromosome reduction, because, if one of the divisions in endomixis is reductional, then there must be somehow, somewhere, a compensating mechanism, for which there is no cytological evidence and against which there is much physiological evidence.

Erdmann from studies on size variation after endomixis in Paramecium has found that variations arise, some of which are heritable.[15] These, however, apparently fall in the same category as the variations which Jennings and his colleagues have demonstrated in pure lines of various Rhizopods, and which are almost surely independent of segregation and recombination phe-

[15] Erdmann, "Endomixis and size variations in pure bred lines of Paramecium aurelia." *Arch. f. Entw.-mech.*, 46, 1920.

nomena as understood in the metazoa. In short, the physiological effect, so far as rejuvenescence is concerned, common to both endomixis and conjugation must be sought in intracellular changes independent of those generally interpreted as following from genetic factors.

The universal method of reproduction in the Protozoa, as, of course, in final analysis in all organisms, is cell division. But while in higher animals reproduction is usually associated with fertilization, in the Protozoa there is clearly presented the essential separateness of these processes since when fertilization occurs it merely punctuates a longer or shorter series of independent organisms which are the products of fission. Fertilization, however, is apparently neither of such universal nor frequent occurrence in the Protozoa as was formerly believed to be the case. Following the early observations of Dallinger and Drysdale on the life history of Monads and the discovery of fertilization in the Infusoria, it was confidently predicted that only further research was necessary to reveal it in all forms. And Schaudinn's beautifully conceived and executed studies on the life histories of a series of Protozoa from Endamoebae to malarial organisms seemed to justify the idea. But recent and intensive work on the Sarcodina and the Mastigophora has signally failed in many cases of the former and most cases of the latter to supply the critical evidence of synkaryon formation necessary to pronounce fertilization a fact, and it certainly must be left an open question whether it is not the exception, rather than the rule, in these classes. At all events, and this is the point of immediate interest, asexual reproduction is obviously adequate in the Protozoa, as it apparently is in certain, at least, of the Protophyta, to carry on the stream of life uninterrupted. It is yet premature to attempt to trace the evolution of sexuality in the Protozoa, but if one may be permitted to hazard a guess, it will prove to be of diphyletic origin and from some nuclear reorganization processes endomictic in nature.

Enough has been said to recall to mind the rapidly changing picture which the so-called dynamic aspect of the problem of fertilization has presented since the pioneer work on conjugation in the Protozoa. Its investigation has been fostered very largely by the belief that the key to the fertilization of higher forms must be sought in the conjugation of unicellular organisms; and the Infusoria, it is hoped, have not so far proved disappointing, when we bear in mind that they have had as long an evolutionary history as man and exhibit in their *multum in parvo* plan of structure a complexity as real as it is different.

THE FOOD OF GROUND SQUIRRELS

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So much of the territory of Western North America is the natural habitat of the ground squirrel and so greatly have they increased with the expansion of cultivated areas and so vast is their destruction to growing crops that a study of their food habits and an exact determination of the damage produced by them is of interest and importance. These animals are prominently represented by one genus, *Citellus,* of which there are many species, enjoying a wide range over the western half of the continent. The species upon which this study is based is the Columbian ground squirrel (*Citellus columbianus columbianus*) found in Eastern Washington, Eastern Oregon and Northern Idaho. Its life, though modified by unusual climatic conditions, is probably representative of the genus.

Observing these squirrels in the fields from day to day during the time of the harvest of their wild grasses and lupines, one is impressed with the aptness of the old generic term *Spermophilus,* which expressed so fittingly one of the chief characteristics of these little seed harvesters. It is a fascinating sight to see them, standing erect among the ripening vegetation, bear-like, gleaning their harvest with capable fingers and teeth. In season, purple lupines, the little rock clover (*Erodium*) and waving seed-ladened bunch grass (*Agropyron*) fill their fields with plenty. Here they toil through the hours of early morning and late afternoon sunshine, busily, in anticipation of the very urgent, present need of the all-important nourishment against the near approaching time of estivation. Not that they are filling, for themselves, their hidden treasure houses, but that they may store up the necessary fat as a body food reserve which

must carry them past the long months of estivation and hibernation. With the return from hibernation they begin feeding upon green vegetation and bulbs of wild plants. This desire for green food increases during the growing season and is given over only when seeds of wild grasses begin to mature.

We have found that foods useful to them at some seasons are not at all so at others. For example, while green substances such as growing grain, alfalfa and wild plants as the balsam root (*Balsamorhiza*) are eagerly devoured while succulent and in the season of early spring, these same plants seem to be of no value to them at a season of maturing grains, even should their growth have been retarded or development delayed by late planting. In captivity, squirrels whose estivation and hibernation has been interrupted do not touch young succulent winter wheat in their yards in the autumn. At this time they are fond of sunflower seed or carrot which they eat in small quantities.

Use of Water

That this animal does not use water directly as drink may be attributed to many of the remarkable phenomena associated with its life history as the early estivation and correspondingly early return to activity from hibernation. That it prefers succulent vegetation to water accounts for the great damage done by the ground squirrel in growing grain, as is later shown in this paper.

Squirrels of this species were observed day after day, in the wild conditions, sometimes in a brookside pasture, but only once during the five or six years of this close observation were they seen to drink, and then there was some doubt about the observation. Many times they have been reported as "abundant in lowlands where they are attracted by water," but the fact is they were attracted there primarily by the green grass rather than by the water.

It was desired to see if this squirrel would drink water. Accordingly three females, in a large yard in which they

were being observed, were given a glass dish a few days previous, so that they might become accustomed to the vessel. On the 28th of July, in the midst of a very dry time, they were given sunflower stocks and eagerly ate out the juicy hearts of these. On July 29, they were given nothing in addition to the natural food in the yard, which was very dry and parched. On July 30, the dish was filled with water. In a very short time one of the squirrels came out of its den and after looking about casually, went over to the dish and at once began to drink. She drank for a long time, lapping up the water quite as a cat would lap it, but with her tongue moving much more rapidly. After she had quenched her thirst, she went away and her place was shortly taken by the other two squirrels, who also drank as she had done. A like test was given young squirrels in another yard. One of them tasted the water, but they seemed to be expecting their food, and almost immediately began to eat carrot when it was given them. On July 31, the old squirrels were given the same dish with water renewed in it, placed in front of the hole as before, and about a foot away on the opposite side of the dish from the hole were placed two carrots, in such a way as to make it necessary for the squirrels to go past the water to reach them. Presently one came out. She sniffed the water, hesitated, then sniffed it again and went on to the carrot. The second one followed her example. The third one did not appear. That they do nicely without water is shown by the fact that they lived for two years in these yards without it.

FOOD MANIPULATION

The handling of food is very dextrously done by the ground squirrels. One was observed in the yards eating buds of balsam-root. It first nosed into a bunch of leaves, selected a half-grown bud, nipped it off and ate most of it. It then reached up and took a whole head in its mouth and with a sudden jerk detached it; then holding the head in its paws and standing quite erect so as to see

well above the grass it began eating, devouring petals and all. When eating clover, they have been observed to seize the blades of the leaf in the mouth and the leaf stalk in the paws and pull until it breaks from the stem.

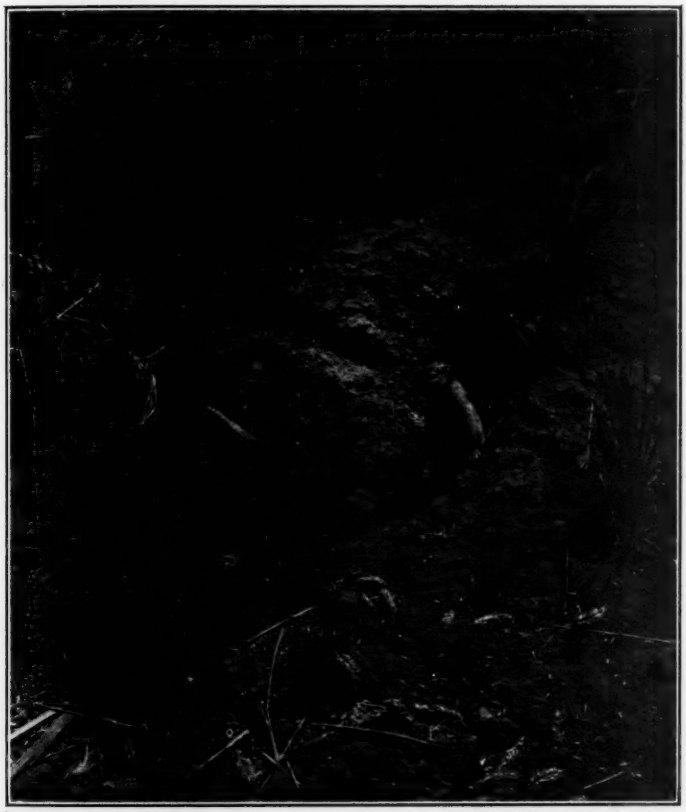

FIG. 1. Columbian ground squirrel (*Citellus columbianus columbianus*) in the midst of a wheat field. The heads of wheat scattered about have been deprived of the grain, yet are left intact.

Not infrequently the squirrel has been observed holding the stem of a clover head in one paw until it had finished devouring it, or even walking on three legs and carrying food in one paw. In gathering clover or other seed-bear-

ing heads they grasp the stalks first and bend them down, often nipping them off and eating down the stalk until they get to the head.

They harvest the grain from the hulls of wheat and oats, leaving the heads so cleverly intact that one is deceived into the belief that they are still full of grain (Fig. 1). Their skill in securing parts of the stock is no less marked.

In the early season the squirrel feeds largely upon grass. It is especially fond of blue grass, and I have observed it in the yards seize a short blade of previously pastured blue grass between its incisors and pull straight up. Finally the blade would break from the crown and be worked back into the mouth by the process of chewing.

A very common squirrel-like attitude while feeding is sitting on the hind feet, tree-squirrel-like (*Sciurus*), manipulating the food with both front paws. Occasionally, when seated on their feet eating, they give the tail a flicker (only one flicker) apparently as an indication of pleasure.

This species of ground squirrel possesses the ability of extracting the tender stems of grain from the stock with a great deal of skill. It is done in this way: From about the 10th of June until the grain has formed it bites through the sheath on the side of the stock a few inches from the ground above a joint and very cleverly pulls out the tender, juicy end of the stock, leaving a tangle of wilted stems to mark the neighborhood of the den. So completely do they take the standing grain that scarcely a stock is left within a rod about and the grain in the vicinity of the holes forms a matted tangle of dead, shattered and crinkled stems adhering by shreds to stubble not yet dead (Fig. 2). From this tangle a second growth frequently springs and slowly develops in a new though unmaturing crop after the squirrels have gone into estivation. By July, when maturing grain finally renders the stocks of wheat or oats too dry for its purpose, attention is given to the ripening grain itself. At this time, when

the grain is too high to reach, it cuts the stock off sharply or crinkles it and drops the head to the ground. It is not uncommon to find grain cut in this way two or three rods in from a fence row, as if pastured by cattle.

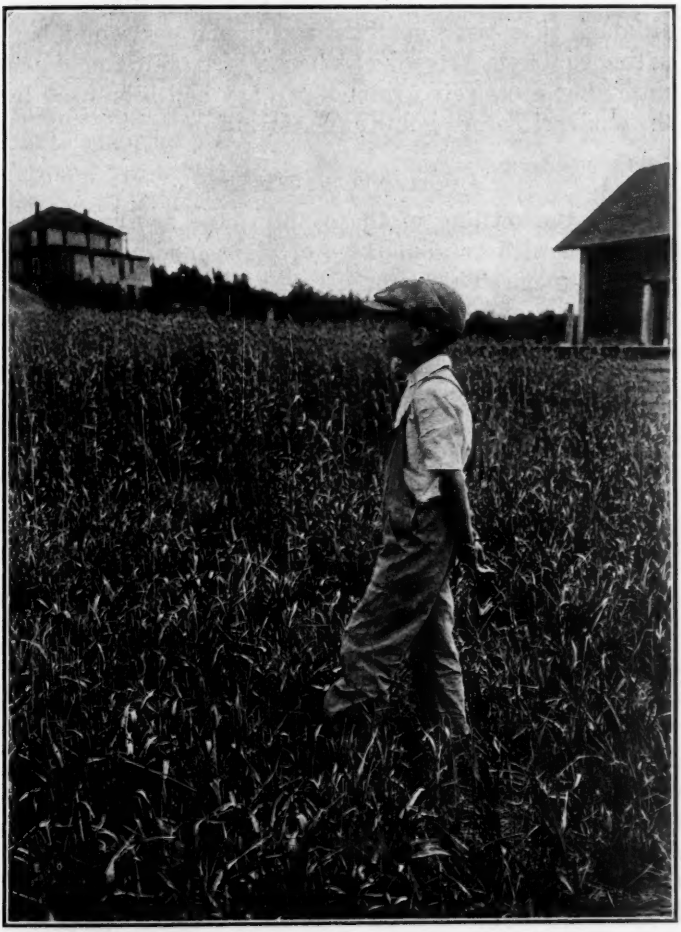

FIG. 2. Devastation in the squirrel-infested yard. The boy, four feet one inch tall, is standing where the stems have been cut since May 15. There is scarcely a head left for many feet around. Compare with Fig. 3. Picture taken July 1, 1919.

STORING OF FOOD

One might expect that these animals would store food for adverse conditions, but in no instance in the numerous investigations of the summer dens of this animal has there been found any trace or suggestion of stored food, although it is occasionally found in the nests of hibernating squirrels. Heads of wheat were rarely found and were almost entirely grainless. They had been used by the squirrel before going into estivation.

CULTIVATED VEGETATION

With the settling of the country, the squirrels were furnished with an abundance of plant food and at once adapted themselves to the use of such cereals as wheat, oats, barley and blue grass. They have a special liking for such legumes as clover and alfalfa. In the latter part of June and through July, 1910, it was noted that the squirrels were feeding upon bean plants, wheat, new potatoes, the juicy stems of corn stocks, the foliage of field peas, carrots and apple.

In the yards, the squirrels have shown a preference for carrots above other foods. Likewise, when they live near an orchard, they will go considerable distances for apples, especially in the spring when they secure the fruit which has passed the winter on the ground under the snow. These they carry to the mouth of their burrow before eating.

STOMACH ANALYSIS

A more exact determination of the food was secured by examining the stomachs. In preparation for the work, specimens were taken at various times of the day from June 10, until July 28, over a wide range about Pullman, Washington. The specimens were collected from such situations as stubble fields, wild oat patches, old pastures, orchards, wheat, oat, barley and pea fields, alfalfa fields, white clover pasture and unplowed fallow. It was a difficult matter to determine specifically the

kinds of vegetable matter eaten, owing to the thorough mastication to which it is subjected. This is also true to some extent in the few insects and larvae found, for they appear to remove the skin, legs and mouth parts, eating chiefly the fat and muscle.

In general, the food of spring and early summer as shown by this study consisted of bulky, fibrous vegetable material such as would be found in grain leaves, grass and alfalfa, while later in the season the stomach contents were less bulky, but more concentrated in finely ground grain products. Animal and vegetable contents of the 43 stomachs examined at different times through the active season appeared in the following percentages:

100 per cent. contained vegetable matter.
86 per cent. contained vegetable matter entirely.
2 per cent. contained traces of mammal.
13.9 per cent. contained traces of insect remains.

The very low particles of insect remains sometimes found would lead one to believe that they were taken accidentally with other food, as in the case of lepidopterous scales found. Those insects clearly recognized in all these stomachs were three ants and two larvae of a carabid beetle (*Calosoma* sp.). One stomach contained some hairs, probably those of a mouse.

Wild Vegetation

When squirrels come from hibernation, they may often be tracked in the snow to the whorls of yarrow leaves (*Achillea*) exposed by the sun, or they soon begin digging in the moist earth, probably securing bulbs of wild plants, such as Allium. There were signs of their having eaten buttercup blossoms, growing near their burrows, and later in the season wild oats were used by them. Strange to say, the bunch grass blades so common in their territory seemed of little use to them even when green and young and were resorted to as food only when other forms of vegetation were lacking in the yards. When driven by hunger, however, they would eat them.

The form of blue grass (*Poa* sp.) growing in the yards was eagerly sought. This they kept cropped down closely. They were fond of the buds of the wild roses and kept the leaves and blossoms nipped off some distance from the ground. Wild lettuce (*Lactuca*) was eagerly devoured by them. It was quite impossible to start clover of any sort where the squirrels were kept.

AMOUNT OF FOOD PER DAY

In conducting the experiments for this determination, the squirrels were fed as usual the day before. The night before the test the yards were carefully cleaned of all possible food and everything put in readiness for the beginning of the test early in the morning. To account for evaporation and the loss by weight from this source, checks of an equal amount of food were put in adjoining yards under exactly similar conditions and weighed each time the test was made. This, taken from the total loss of weight of the amount given the squirrel, will give the amount eaten. These tests were commenced early in the morning before the squirrels came out and were continued until late in the evening to make sure that they were through feeding.

The test of May 14 shows the following results:

1ST TEST, 6:30 A. M. TO 9:00 A. M.

Clover	4,067 grams
Clover check	4,067 "
Apple	57 "
Apple check	57 "
Clover eaten	9 grams
Apple eaten	9 "
Total	18 grams

Subsequent tests, using the same methods and material at 9:45 A. M. and 2 and 4 P. M. gave 8, 44, 21 and 44 grams, respectively, as the amount eaten, making a total for the day of 135 grams.

This test was carried on with a female, the mother of a family of young. At the beginning of the test she weighed 405 grams, the young gained nine grams, weigh-

ing 244 grams at the end of the test. The female consumed 33.3 per cent. of her weight.

In order that this test should be as natural as possible the clover was given as sod and was placed on a large glass and tin plate so that no dirt would be lost. Squirrels do not readily eat wilted grass or clover.

A similar test was made May 16, with the same squirrel, using wild white clover alone. On this date she weighed 412 grams and consumed 71 grams, or 17.2 per cent., of her weight of the clover from 6:40 A. M. to 7 P. M. Her four young were 21 days old and weighed 266 grams.

In a similar manner it was found that a young squirrel 4 months old, weighing 237 grams, consumed 106 grams of carrot on July 31; 71 grams on August 1; 100 grams on August 4, and 89 grams on August 5, being fed carrot exclusively during these tests.

The Problem of Squirrel Cost[1]

That the Columbian ground squirrel was very costly because of its injury to growing crops had long been recognized, but the actual amount of damage suffered had not been measured. An investigation was started, therefore, in October, 1918, for the purpose of ascertaining the damage done by squirrels to winter wheat. Positive measurable results were secured and are here reported.

The Method of Study

To further this investigation, a typical piece of fallow Palouse wheat land was selected and sown to a good variety of wheat. The weather being favorable, the wheat germinated promptly and soon covered the ground with a good stand.

Before winter, a part of this land was divided into sections 50 by 50 feet and a retaining fence three feet high and two feet in the ground was constructed about each plot to make it possible to hold the squirrels in absolute control. Within each of these plots was an old surface-obliterated squirrel den, such as is common in cultivated

[1] Popular Bulletin No. 118, Washington Experiment Station, Pullman.

fields of the Palouse, even after the squirrels have been absent from them for many years. In these dens were many burrows too deep for the plow to crush. Once reached by the squirrel they were easily and quickly renovated and made into an inhabited dwelling, with plenty of wheat at hand. Immediately adjoining were check plots of similar size, slope and soil.

The following spring all plots were cut back to 40 by 40 feet for an even stand, away from the fence. Wild squirrels were trapped, branded and otherwise carefully marked and recorded in preparation for the season's test and were placed in the enclosed plots as described below. The behavior of a representative male squirrel designated as Male No. 1 will be given in some detail; the observations made in regard to the others more briefly.

Male No. 1 was placed in an old den underlying the wheat plot in Yard 1, on March 22, at a time when the wild squirrels were beginning their work on the winter wheat in the fields. On the following day it began eating the succulent young leaves of the wheat. This it kept up steadily until the seventh of May, but the general detrimental effect upon the wheat was not noticeable other than the gradual thinning around the den. By May 9, however, a very marked, sudden and disastrous change had taken place. The wheat leaves were becoming less succulent and the squirrel, representing a species which is not dependent upon water for its liquid nourishment, had suddenly attacked the stems of the wheat plants and in the brief time elapsing had strewn the ground with wilted leaves and broken stems. In doing this it cut very skilfully into the side of the stem just above one of the lower joints and extracted a small bit of the tender juicy stem. Stem after stem had been cut to the ground and with each stem went the loss of one future wheat head. It is at this time that the wheat suffers its greatest devastation from the squirrel; for, where earlier a few bulky leaves and later an equally small number of concentrated heads would satisfy a squirrel's hunger, now it would take a large number of these very short tender stems to

make a satisfying meal. From this date until May 27, the squirrel worked continuously upon the stems without variation, except that on May 23 it began cutting higher up on the stock in regions of more recent growth (Fig. 1).

On June 27, a change took place. The stems were now becoming woody in anticipation of approaching harvest. The squirrel abandoned its work on the stems and began feeding on the maturing grain. By this time the region about the den was well carpeted with a mat of dead leaves and stems.

In order to reach the heads of wheat the squirrel cut into the side of the stem up about the third joint, sometimes the fourth, usually just below the joint. In this way the stem was crinkled and the head dropped to the ground, and a matted tangle of wheat stems soon replaced stalwart waving stocks (Fig. 2). Many times the stem was completely cut off. In most cases the heads were then cut from the stem, close up to the grain, and carried to the mouth of a burrow and there the grain extracted and eaten. So skilfully was the grain separated from the head that the latter was left quite intact. By July 30, when the wheat was harvested, the squirrel had finished the depredations, accumulated sufficient fat for the long period of estivation and hibernation and was seen no more. The immediate vicinity of the den was strewn with empty wheat heads, and for nearly a rod around the ground was carpeted with bleached leaves and stems. From the area of the May and June stem cutting, now some green second growth heads struggled through, too green to mature and too short for the reaper.

The Result

On July 30, the several plots and their corresponding checks were harvested, the differences taken and the damage carefully calculated. It was found that during the 130 days of activity, from March 22 to July 30, the squirrel in Yard 1 had destroyed 44 pounds of wheat. Equal areas of 1,600 square feet were harvested, one squirrel infested and one free, showing in grain 89

pounds against 133 pounds, respectively. As a more striking comparison 500 square feet were harvested in the immediate vicinity of the den in Yard 3 (Fig. 2) and

FIG. 3. A normal stand of winter wheat in a check plot adjacent to the plot illustrated in Fig. 2. Photographed under exactly similar conditions.

an equal area in the check plot for this yard (Fig. 3). The squirrel-infested area produced 4 pounds of grain against 41 pounds in the squirrel-free area (Fig. 4) and

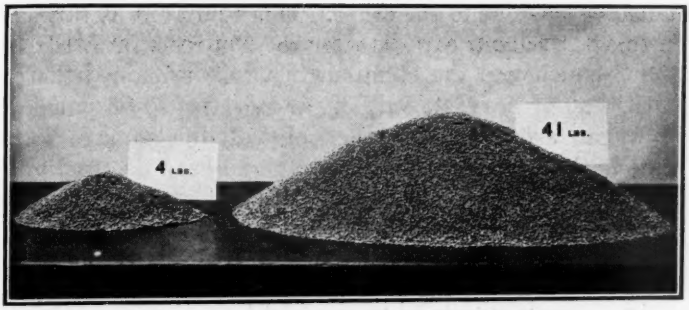

FIG. 4. Wheat secured from check plot and squirrel-infested plot. Forty-one pounds of wheat were secured from 500 square feet of the former as compared with four pounds of an equal area of the latter.

in straw, 1 sheaf against 9 (Fig. 5). This does not represent the entire damage done by the squirrel in Plot 3, as much was done at a greater distance from the den. A second test was made with a family of squirrels working on a 40 by 40 foot plot of wheat in Yard 2. Here the amount of wheat destroyed was 51 pounds.

In a third test, a male squirrel, Male No. 2, was placed in Yard 3 on May 15 to determine *when* the greatest

FIG. 5. Straw secured from check plot and squirrel-infested plot. Nine sheaves were harvested from the former, as compared with one from the latter. The retaining fences and plots are also shown.

damage was done to the crops. This squirrel was turned in this yard about a week after the squirrels in Yards 1 and 2 had begun the stem cutting. He remained there until time of harvest, July 30, and destroyed 56 pounds of wheat, showing that practically all the damage was done in the 76 days of stem-cutting and head destruction. The average destruction of winter wheat per squirrel per season in the experiments described therefore was one third of 44 plus 51 plus 56 pounds or 50 1-3.

GROUND SQUIRRELS AND PASTURE

Squirrels are very destructive to pasture and alfalfa, although their work is not noticeable to the casual observer as it is in grain. As already noted, one squirrel destroyed 71 grams of white clover in one day. At this rate, 385 squirrels would consume the pasture of one cow in a day or 96 squirrels would devour the pasture of one sheep in a day.

These tests were carried on under fairly normal conditions. It is true the squirrels were restricted to wheat and to whatever other plants were incidental to average wheat lands. It is also true that many squirrels live in exactly the same situations out in the center of large areas where they would have no other food.

NUMBER OF SQUIRRELS PER ACRE

During seven successive years, 7,000 squirrels were trapped on 416 acres of the college farm, or 2.4 squirrels per acre each year. A successful wheat raiser near Steptoe, Washington, trapped 1,200 squirrels on 200 acres in one year, or 6 squirrels per acre. This gives a fair estimate of the abundance of these animals.

In places where they are not controlled, they become very numerous and may destroy the entire crop. In an experiment on complete eradication conducted in one wild side-ravine pasture of 25 acres, adjoining wheat land, a careful estimate of 250 squirrels, or ten per acre, were destroyed by gas. Another area of old blue grass sodded bottom land showed an estimated concentration of about 25 squirrels to the acre.

THE EVOLUTION AND DISPERSAL OF THE FROGS

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OUR present conception of the natural groups of frogs and toads has been the outgrowth of many years of investigation by such scholars as Cope, Gadow and Boulenger. In 1882 Boulenger utilized in his classical "Catalogue" a modification of Cope's system (1864–1865). The structure of frogs soon came to be better known and as might have been expected, certain genera were found which did not fit well into Boulenger's groupings. From 1882 until the present day herpetologists have continued to add to our growing information, but not until 1916 was any radical change suggested which met with general approbation. Various authors contented themselves with stating that Boulenger's system was not expressive of genetic relationships, and some such as Gadow suggested minor changes. Boulenger in his last general work (1910) saved the trunk of his old tree by merely lopping off some of its most shattered branches. Recently I have had the occasion to bring together the data which have been accumulating during all these years and have tried to arrange this material in such a way that it would accord with the known facts of anatomy, paleontology and zoogeography (Noble 1922). Before this could be accomplished some preliminary papers by Metcalf (1920, 1921, 1921a) appeared, claiming that the origin and dispersal of the families of Salientia may be determined by a study of their opalinid parasites. These parasites Metcalf plainly tells us are in general not specific, one species frequently occurring in many different genera of frogs, in different families and even in salamanders and fish. The fact that the same species of opalinid was found in two frogs did not show that these frogs were related, for Metcalf tells us that reinfections of the parasite have oc-

curred many times. I failed to see that Metcalf's data had any bearing on the past migration routes of the frogs and toads and so stated in my earlier paper (1922).

Since this time Metcalf (1923) has published his data in full. This detailed account confirms my earlier conclusions, and there would be no occasion for discussing the subject further were it not for the fact that Metcalf (1923a) has reiterated his claim in one of the recent numbers of this journal.

The evolution of the various groups of frogs and toads has been determined chiefly by a detailed study of their osteology, but all anatomical data, especially the myology, have been considered. The fossil evidence is much too scanty to be of value, but as there has been found a general agreement between the osteological, myological, ontogenetic and zoogeographic data there can be little doubt that the evolution of the group has been sketched correctly as to its main features. Metcalf, however, finds it convenient to fall back on some of the text-books which are admitted to be inadequate by all students of the group. In short, many of Metcalf's families of Salientia are known to be artificial, but this has not prevented him from making numerous broad generalizations in regard to their origin and dispersal.

The most primitive of living or fossil frogs are the *Discoglossidae,* and of these *Liopelma* and *Ascaphus* stand nearest to the labyrinthodont ancestors of the order. These two genera, widely separated geographically, agree among themselves in such fundamental features as the retention of a single coracoscapula (in addition to the "coracoid"), without acromion, the presence of a large pubis ("prepubis") and the presence of 9 presacral vertebrae which are not opisthocoelous—as usually stated—but really amphicoelous, for the intervertebral cartilages ("centra") never ossify nor do they more readily attach themselves to the vertebra immediately before than to the one behind. *Ascaphus* and *Liopelma* are unique among frogs in retaining two tail-wagging

muscles (caudalifemoralis and caudalipubois chiotibialis), although neither possesses a tail. Further, *Liopelma* retains 3 pairs of cartilages lying in the inscriptio tendinea of the rectus abdominis, which because of their enormous size (nearly as large as the sternum), may very likely prove to be remnants of the ventral armature of labyrinthodonts and the gastralia of *Sphenodon*. From these primitive discoglossids the other genera apparently arose by the development of true opisthocoelous vertebrae and by the modification of pectoral and pelvic girdles. Other changes may be traced in the myology and especially in the urinogenital system. Although these modifications within the discoglossids are the subject of a study still unpublished, it may be mentioned here that *Ascaphus* retains as evidence of its lowly position both post cardinals, exhibits no fusion of Müllerian and Wolffian ducts in the male, and has a kidney which extends the entire length of the coelom. From some of the higher, purely aquatic discoglossids the *Pipidae* (Aglossa) have developed merely by an accentuation of those changes which have occurred as the extreme modifications of other aquatic frogs. A detailed statement of these changes have been given elsewhere (Noble 1922). The higher groups of frogs and toads have evolved from discoglossid or pro-discoglossid ancestors in somewhat divergent lines. The *Pelobatidae* form a natural group of primarily terrestrial or fossorial forms. The brevicipitids and ranids form a second natural group, the hylids and bufonids a third. In each of these three stocks of higher frogs various lines of descent may be traced. Certain changes have occurred within these lines which parallel similar modifications in other lines. Of the more conspicuous changes may be mentioned the reduction in the number of vertebrae, the fusion of the two halves of the pectoral girdle in the midline and the loss of the maxillary teeth. These lines of descent are not merely theoretical—drawn on paper by systematists—they are natural lines as shown by the sum total of ex-

ternal and internal characters as well as by the life history and distribution of the groups. The picture is complete for a large series of lines, but is still fragmentary for others.

When these lines of descent were grouped according to their natural affinities the question arose, Did the present distribution of either the larger or smaller categories demand the existence of former transoceanic land bridges to account for their distribution? In every family it was found possible to assume a holarctic dispersal, except for one recently evolved group, the brachycephalids, which still reside in their ancestral home, the neotropics.

Metcalf believes that his opalinid data give an important clue as to the routes of migration followed by the Salientia. Let us, then, examine his evidence in more detail. Passing over the discoglossids which Metcalf agrees arose in the holarctic regions we come to their relatives the pipids and find that unfortunately the American representatives of the family are not known to be infected by opalinids, and hence Metcalf's conclusions in regard to this family are pure assumptions. Turning to the pelobatids, Metcalf (1923, p. 361) tells us that "the opalinid parasites of this family throw little light upon the problem of its geographical distribution." And what information does he give us as to the higher families? The "Bufonidae are an Equatorian family" he writes (1923, p. 364), "dating back to the Triassic period." But Metcalf has studied the opalinids of only two genera! One of these, *Engystomops,* is not a "bufonid" at all, but a toothless derivative of the "leptodactylids." *Paludicola,* with which it agrees not only in osteological detail (witness the peculiar sternum, common to both), but also in regard to the essential features of its specialized life history (the eggs develop in a foamy mass of spawn in contact with the water). The other genus, *Bufo,* Metcalf (1923a, p. 403) admits "adopts any opalinids present in its habitat." Metcalf has not been fortunate in securing

infections from any critical genera or families. He had before him not a single opalinid from any "dyscophid" (toothed brevicipitids), the ancestral stock from which all "gastrophrynids" were derived. Still, we should not discuss the latter family, either, for Metcalf (1923, p. 369) tells us that "the Opalinid parasites of the *Gastrophrynidae* do not throw much, if any, light upon the problem of their distribution." In short, Metcalf's data can be applied to only three families: the ranids, the toothed bufonids (leptodactylids) and the hylids. In regard to the first it is universally recognized that the oriental and African ranids are allied, and that *Rana* must have had a northern center of dispersal, but there is no evidence "that *Raninae* have lived in northern South America apparently since early Cretaceous times." These so-called *"Raninae"* are close relatives of the toothed bufonids. And they agree with South American bufonids not only in the critical features of their osteology and myology, but also in their opalinid parasites. They differ from bufonids in their solid pectoral girdle, and are therefore classified as a distinct family, the *Brachycephalidae.* Some brachycephalids still retain a partly arciferal girdle as definite evidence of their bufonid ancestry.

In regard to the "leptodactylids," Metcalf has nothing to add, save that certain opalinids and these frogs happen to be found in the southern hemisphere. Now the phylogeny of many genera of leptodactylids has been worked out in detail. It has been found that the end forms were almost invariably without maxillary teeth. Wherever in the world toothless "leptodactylids" (bufonids) occur it may be readily assumed that they have arisen from toothed forms. In certain regions, as in India, the toothed forms no longer exist, but the toothless genera are there as evidence that toothed forms either existed in this region or an adjoining one. Toothless bufonids can not be separated as a family from the "leptodactylids;" we must necessarily widen our conception of the

family *Leptodactylidae*. When this is done it is found that the "leptodactylids," or, as we now call them, bufonids, have a world-wide range, and lend no support to the theses expressed by Metcalf.

We come at last to the one remaining family, *Hylidae*. The occurrence of *H. wachei* in Africa makes it difficult to assume a southern origin for the group. But Metcalf is attracted by the present hiatus of range in the Indo-Oriental region. He concludes (1923a, p. 394) "It seems wholly unlikely that Hylids, whether of Nearctic or of Palearctic origin, could have entered Australasia from the north without leaving Euro-Asia and Malaysia species, showing affinity to present Australasian tree frogs." Would Metcalf then have us believe that extinctions rarely occur? In any group where the fossil material is well known, as in the mammals, we have abundant evidence of such extinctions. Fossil Salientia are rare, especially well-preserved material. Recently it has been my good fortune to have placed in my hands for description a beautifully preserved pelobatid from the Oligocene of Mongolia. Here is a case of where we have definite proof of the former existence of a family in an area where it does not now reside.

It must, nevertheless, be admitted that the distribution of the *Hylidae* presents the weakest point in the evidence which I have brought together. And although Metcalf has added nothing to prove or disprove the theses submitted, his theories have recently received support from an unsuspected quarter in the able herpetologist and zoogeographer, John Hewitt (1922). While admitting the classification brought together by me to be a natural one, Hewitt would prefer to assume that the radiation into Asia, Africa and South America came, not from the north along land masses existing to-day, but from the south on three *theoretical* land masses existing in Mesozoic times. Antarctica, to be sure, enjoyed a temperate climate in Jurassic times, but proof that this land mass was connected with either South America, Africa or

Australia is still lacking. We have the fossil evidence that the mammals have migrated along existing land masses all over the world in Tertiary times. Surely the Salientia did not remain stationary during these 60 millions of years! Witness the flood of northern frogs (*Rana, Bufo, Gastrophryne,* etc.) which have entered South America since the Panama connection. The story of these wanderings is by no means complete as to detail, but all evidence points clearly to the fact that the migration routes lay along existing continental masses. Perhaps we can not say that the lowly toad accompanied the camels or marsupials in their wanderings, but at least they trudged along the same roads.

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PHYLOGENY IN THE ORIBATOIDEA

ARTHUR PAUL JACOT

In an order of animals which are outstandingly predatory or parasitic, as, for instance, the Ixodoidea, which are parasitic chiefly on vertebrates, the Boellidae, which are predaceous on tiny animals, the Cheyletidae, which are both predaceous and parasitic, the Tetranychidae, which are chiefly suckers of plant juice, the Erythraeidae, which are chiefly suckers of animal juice, the Trombidiidae, which are parasitic as larvae and predaceous as adults, the Gamasidae, Sarcoptidae and others, we find one family which is distinctly herbivorous and non-injurious or saprophytic, filling a similar though far less conspicuous place in nature as do the ungulates and rodents among the mammals. They browse on vegetation or feed on decaying plant or (much more rarely) on decaying animal matter. In a group, the Acarina, which is characteristically positive, aggressive and well supplied with organs of attack, we find this one group, the Oribatoidea, which is negative, humble and devoid of weapons of offense.

Since in nature it is necessary for inoffensive animals, in order to maintain themselves on the surface of the earth, to be protected from the predaceous, it is interesting and instructive to consider how this isolation group is protected.

Although one of the chief characteristics of the Arthropoda is an external skeleton which acts as a coat of mail on the exterior of the body, the Acarina and Araneae have this dermal armature far less developed than is the rule in the majority of the other orders. An analysis of the various families and superfamilies of the Acarina for the purpose of comparing the amount of chitinization gives the following results:

Demodecoidea—very thinly chitinized.
Eupodoidea, Sarcoptoidea, Trombidoidea—soft and delicate.
Hydrachnidae—usually soft, sometimes with dorsal shields, more seldom uniformly chitinized though not strongly.
Halacaridae—with coriaceous plates or shields or entirely leathery.
Ixodoidea—fairly well chitinized, skin coriaceous to leathery.
Parasitoidea—with coriaceous plates and shields.
Oribatoidea—body quite coriaceous to highly chitinized.

The Oribatoidea, therefore, stand out from among their phylogenic relatives by reason of the extreme amount of chitinization.

A more detailed study of the group will, however, show that all the species are not so highly chitinized. Species, to be more specific, belonging to the genera *Hypochthonius, Nothrus, Neoliodes* and their related genera are quite as poorly chitinized as are species of other poorly chitinized superfamilies. In the genera *Scutovertex, Cepheus, Hermannia, Tegeocranus* (using these terms as did Michael) and their related genera one finds species much more fully chitinized but with the general appearance of the *Nothrus* group, while in the genera of the Eremaeinae, Oribatinae, Pterogasteridae and Ptyctima we find highly chitinized, smooth to glossy species, which are the climax forms for this one protective character. As a smooth, glossy sphere is more difficult to cut into than a roughened, angular form, the genera having reached this stage may be considered more advanced than the others—other things being equal.

A survey of the many forms of non-predaceous animals for the purpose of determining their various means of protection from enemies, besides that of exterior armor, will recall flight, development of special structures of rebuttal (as horns), leaping and retractility of soft parts within or behind hard parts or areas as in the foraminifera, bryozoa, brachiopoda, mollusca, barnacles, cladocera, turtles and others. Among the Oribatoidea three of these forms have been employed with apparent success.

Species of the subfamily Eremaeinae have recourse to flight and consequently have developed strong, fairly long legs (compared with the Hoploderma, Hypochthonii,

Nothrii, etc.), each segment being broad, bulby, heavily muscled and articulated so as to give each a maximum of flexibility. In the Oribatinae the legs have become very much over-developed in relative length of each article so that the legs are insufficiently muscled to warrant rapid action. As a result, in animals which seem to be built for great speed, one finds slow, sluggish progress, much as in the herons, storks, pycnogonida, phalangoids, etc. In these forms the body is medium to small in size and not specially protected except by the chitinization. These species are developing into legs (carried to extreme in the Pycnogonida) for no good reason except that they are mutating in that direction. This group, then, is a climax group, marked by its highly chitinized exoskeleton and the extreme development of leg segments, either in girth or length, in comparison with the size of the body.

Species of the subfamily Zetorchestinae have developed the ability to leap when disturbed, by the development of the proximal segment of the hind leg, much as has happened in several groups of Arthropoda. They also may be considered a climax group in one of the major groups.

The third method of protection that is used by species of Oribatoidea is that of retraction of vulnerable parts within or behind hard parts. This has been accomplished by two clearly defined and separate methods. In the Ptyctima the upper posterior edge of the head (cephalothorax) is hinged to the upper anterior edge of the abdomen so that it may be bent downward against the antero-ventral part of the abdomen. Before this is done, however, the legs are drawn into the abdomino-cephalothoracic cavity. Thus the animal presents a hard, oval or flattened oval to its enemy. This, then, marks a climax development as the protection is complete. It might be here added that the subfamily Tritiinae, which I have discussed elsewhere, is more highly developed than the Hoplodermatinae (Phthiracarinae) by virtue of the fusion of the ano-genital plate to form two long and very narrow

plates, and by the extreme compression of the abdomen and consequent narrowing of the venter or ano-genital area. In the Aptyctima the converse, as far as relation of cephalothorax and abdomen are concerned, is found. Protection to vulnerable parts is insured by special leaf-like or plate-like growths or expansions of the chitinous body covering behind or under which the vulnerable parts may be retracted. Of these expansions there are two distinct types. One type, called the pteromorphae, consists of an expansion from each side of the abdomen; the other type, the lamellae and tectopedia, consists of paired expansions springing from the cephalothorax. The presence of the former comprises the family Pterogasteridae, the absence of pteromorphae characterizes species of the other family, the Apterogasteridae.

The Apterogasteridae have been divided into the following subfamilies chiefly on the basis of development of legs. In the Camisiinae the legs are composed of short, squat segments with poor flexibility of motion, thus being much more fitted for exerting great pushing force than for flight. The body is poorly chitinized, rough and dull. Thus they are protected from attack only by their unattractive and inconspicuous appearance when on their habitudinal substratum. The lamellae and tectopedia, which would otherwise give them good service, are absent or insufficiently developed to afford any real protection. Thus, this is the least specialized or most primitive group of the family and should, therefore, be placed first in a linear arrangement. It is from this group that the runners and jumpers have been developed. In the Eremaeinae, as above mentioned, the legs are specialized for running. Their lamellae and tectopedia are of no efficiency and can only be considered ornamental or abortive structures. The body is usually smooth and shining. In the Oribatinae the legs have become very much overdeveloped and the lamellae are still less developed. They thus form the climax of this line of specialization. The Zetorchestinae, as above discussed, would also fall into

this family, having specialized in jumping instead of running. The Serrariinae, consisting of species which have specialized in food habits because of the peculiar development of the mandibles, form the fifth and last subfamily. Nowhere in this family do we find such extreme development of lamellae and tectopedia as in the next. One would not expect to find rapid runners with large and cumbersome lamellae except as one of those overdevelopments which nature occasionally indulges in. The subfamily might be further subdivided into tribes as outlined by Ewing (1917, June), but it would add nothing to the comprehension of the group to multiply the number of subfamilies.

Turning now to the Pterogasteridae, one is bewildered by the large number of genera and the arbitrary way in which they follow one another in systematic papers. A study of the group, however, reveals definite lines of specialization. Aside from the Pelopinae which have specialized (though not as far as the Serrariinae) in food habits and are consequently discriminated by their diminutive mandibles (or, conversely, have specialized in food habits *because of* the mutation of the mandibles), the genera of this family are marked by the greater or lesser development of their pteromorphae, lamellae and tectopedia.

Taking up first the pteromorphae, we find they have become specialized along two distinct lines. In the genera related to the genus *Galumna,* these structures have become very large, passing well beyond the anterior edge of the abdomen but, more important, they have become distinctive structures hinged to the abdomen and capable of being raised or depressed. They thus form enormous lateral wings or covers which, when the legs are withdrawn into the cupboards on each side of the abdomen, close down over the edges of the cupboard and completely enclose the legs and pseudostigmatic organs. Thus the animal is as well protected (except for the mouth) as are the species of Hoplodermatidae. Furthermore, these

species are smooth and shining and the lamellae and tectopedia are very small to practically lacking as such. Thus, the specialization is complete and this as a climax group may be placed in the subfamily:

Galumninae subfam. nov. Type genus: *Galumna* Heyden, 1826. Pterogasterinae having the pteromorphae very large, extending downward to cover part of the ventral plate and forward to cover part of the cephalothorax, legs received in cupboards in the sides of the abdomen and entirely enclosed by the pteromorphae, pteromorphae *hinged* to sides of abdomen, lamellae and tectopedia quite small to lacking as such.

As thus delineated this subfamily would include the genera *Galumnella* Berl., 1916, *Neoribates* Berl., 1914.

Another line of development of the pteromorphae is found in the group of genera which are grouped about the genus *Oripoda.* In these the pteromorphae are also enlarged beyond the anterior edge of the abdomen, but they are not hinged to the abdomen. They are, on the other hand, united about the base of the abdomen and overhang the cephalothorax. The lamellae may or may not be developed (depending on the amount of development of the pteromorphal bridge) and a translamella may or may not be present. This line of special development may be known as subfamily:

Oripodinae subfam. nov. Type genus: *Oripoda* Banks, 1904 (Sept.). Pterogasterinae in which the pteromorphae are very much developed anteriorly and joined to each other along the anterior edge of the abdomen forming more or less of a bridge over the cephalothorax. This bridge may or may not be more or less fused to the cephalothorax. The following genera would therefore be placed therein: *Achipteria* Berlese, 1885, *Gymnobates* Banks, 1902, *Tenuiala* Ewing, 1913, *Jugatala* Ewing, 1913 (of which *Neogymnobates* seems to be a synonym). In none of these genera do the pteromorphae seem to be particularly attached to the cephalothorax. If such should, however, be the case the character would be of generic value only, as it would be secondary to the development of the pteromorphae *over* the cephalothorax.

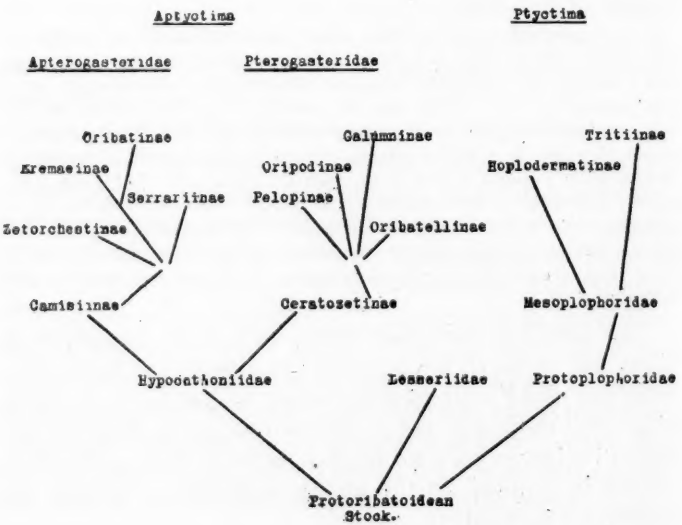
The lamellae have also developed along two distinct lines, one in which the lamellae are attached to the cephalothorax along their posterior edge only and one in which they are attached to the cephalothorax along their mediolateral edge as well as their posterior edge. The first group is typified by the genus *Oribatella,* and may be known as subfamily:

Oribatellinae subfam. nov. Type genus: *Oribatella* Banks, 1895. Pterogasteridae in which the pteromorphae are not developed beyond the anterior edge of the abdomen, but in which the lamellae are attached to the cephalothorax along their posterior edge only and are usually of relatively large size (*i.e.*, covering over a fairly large part of the cephalothorax). It would thus include, among others, the following genera: *Lepidozetes* Berl, 1910 (= *Tegoribates* Ew., 1917), *Joelia* Oudms., 1906.

The remaining genera, characterized by having the pteromorphae small, at least not extending anteriorly beyond anterior edge of abdomen, and by having the lamellae more or less attached by their inner lateral edges to the cephalothorax, and thus presenting no special line of development, may be grouped in the subfamily:

Ceratozetinae subfam. nov. Type genus *Ceratozetes* Berl., 1908. Pterogasteridae as characterized in the above paragraph. This group might further be divided into two tribes based on presence or absence of translamella. As this character is not clean cut, and until it can be shown to be a definite line of specialization by finding species in which the translamella is very much developed or overdeveloped, there would be no point in making a separate subfamily of it.

These groups and their relationships may be more easily visualized by reference to the following diagram:



This relationship, if arranged in linear form, would be: Hypochthonidae; Lohmanii, Nothrii, Tegeocranii in the Camisiinae, Serrariinae, Zetorchestinae, Eremaeinae, Oribatinae in the Apterogasteridae, Ceratozetinae, Oribatellinae, Galumninae, Oripodinae, Pelopinae in the Pterogasteridae; Lesseriidae, Protoplophoridae, Mesoplophoridae, Hoplodermatidae and Tritiinae in the Ptyctima, rather than the arbitrary order introduced by writers early in the nineteenth century. Such an arrangement would present the elements of the group in their natural sequence or order of development and would therefore be a more logical arrangement.

SHORTER ARTICLES AND DISCUSSION

THE HARE-RABBIT, A STUDY IN EVOLUTION BY HYBRIDIZATION

ONE view concerning the method of evolution, which has many supporters at the present time, is that hybridization is one of evolution's principal agencies. This view is usually advocated as an alternative to the view that variations, which form the material for progressive evolution, arise chiefly within the species, by spontaneous variation.

The latter view was held by Darwin, and as an illustration of it he cited the varieties of domestic pigeons, all as he thought derived from the same wild species, the rock pigeon, *Columba livia*. Darwin's interpretation has been challenged in recent years by Ghiji, who has shown that domestic pigeons can be crossed with one or two wild species other than *Columba livia*, resulting in the production of fertile hybrids. This result does not, of course, show that historically the production of domestic varieties of pigeons was actually accomplished by that method, since it may after all have occurred by mutation within the single species, *Columba livia*, with which all varieties of domestic pigeons are fertile. Nevertheless, it establishes the possibility that hybridization *may* have occurred and acted as an accelerator of the evolution of domestic varieties.

Another domestic animal, which has varied perhaps as extensively as the pigeon, and which has figured largely in discussions and experimental studies of evolution is the rabbit. As in the case of the pigeon, the chief, if not exclusive, ancestral species still exists wild, and domestic varieties are fully fertile with it. This is the wild rabbit of Europe, *Oryctolagus cuniculus*.

Domestic breeds of rabbits differ greatly in size, color, ear-length and proportions of the body. The smaller breeds are of about the size of wild rabbits, but other breeds are four or five times as large. This latter fact has led many to suppose that the European hare, *Lepus timidus*, which is much larger than the wild rabbit but similar in appearance, has crossed with rabbits to produce these large breeds, and that genetic heterogeneity resulting from the cross has probably produced further variability among domestic breeds.

The question of possible hybridity between the hare and rabbit has been hotly debated in Europe for half a century both by

practical rabbit breeders and by scientists. Supposedly conclusive experimental evidence has been brought forward more than once that the two species do cross, but has always turned out in the end to be *not* conclusive. The "Belgian hare" purports in its name to be of hare origin, but it turns out to be so only in name, for (1) it burrows like other rabbits, (2) has young born naked and with closed eyes, and (3) is fertile with all other rabbit varieties. On the other hand, hares (1) do not burrow, (2) have young born furred out and with eyes open and (3) admittedly rarely, if ever, cross with rabbits. The burden of proof rests with those who think hares *can* cross with rabbits.

From a personal communication, I gather that Professor Lotsy, who regards hybridization as the chief if not exclusive method of evolution, is one of those who believe that in recent years a skilled rabbit breeder in Holland has actually made under controlled conditions a cross between a male hare and a female rabbit, resulting in the production of fertile hybrids. These admittedly resemble rabbits and produce young hairless and with closed eyes. Yet Lotsy believes them to be genuine hybrids. But conclusive evidence in support of this view has so far as I know not yet been published.

In this uncertain state of affairs, it is gratifying to learn of a thoroughly critical experimental investigation of the question recently made by two Japanese zoologists, the results of which appear to be conclusive. I wish to call attention to it, not only because of its intrinsic merits, but especially because being published in a Japanese journal in the Japanese language (with only a summary in German), it is likely to be overlooked and its bearing on general questions is likely to go unnoticed. The authors are J. Yamane and T. Egashira. The article is found in *Dobitsugaku Zasshi,* a monthly magazine of zoology published by the Zoological Society of Japan, Vol. 36, No. 430, 1924. It deals with experiments, attempted both by natural and artificial means, in crossing the domestic rabbit with the Japanese subspecies of the European hare (*Lepus timidus ainu* Barr.-Ham.). The results were as follows:

(1) Attempts at breeding hares in captivity were fruitless on account of the extreme wildness of the animals, in agreement with all similar attempts previously made in Europe.

(2) Attempts at crossing hares and rabbits reciprocally by natural means were without success. The hare would in no case consent to mate with a rabbit.

(3) A young female hare weighing 540 grams which had been recently captured was put in a pen with a male rabbit of like size, in the hope that the two might be reared together and thus become friendly, but without success. The hare refused to eat until removed again to a separate pen.

(4) An adult female hare was put in a large grassy outdoor pen with a male rabbit but was found next morning bitten to death by the rabbit.

(5) Attempts at artificial insemination of female rabbits resulted as follows. Sperm was taken from the epididymus of male rabbits and after dilution with a 3.3 per cent. solution of dextrose was injected into the uterus of the female. In 3 out of 36 cases pregnancy resulted. In another group of cases the sperm injection followed copulation with a male rabbit, rendered sterile by ligaturing of the *vasa deferentia.* In this group of cases the success was greater, pregnancy occurring in 15 out of 24 cases, or 62.5 per cent. of the trials. This result confirms the earlier finding of British zoologists that in the rabbit ovulation is not spontaneous but is largely dependent upon copulation.

Having now developed a technique satisfactory for successful artificial insemination of rabbits, the authors next substituted hare sperm for rabbit sperm in their experiments, confident that if rabbit eggs can be fertilized with hare sperm, they would be able to produce hybrids.

Sperm in which lively movement could be observed under the microscope was taken from the vasa deferentia of hares, diluted with dextrose solution, and then injected, as in the experiments previously described, into the uterus of a female rabbit which had just copulated with a sterile male rabbit. In 38 cases the result was entirely negative.

In another set of experiments the procedure just described was followed and then from one to five hours later the female was allowed to copulate with a normal fertile male rabbit. In two out of five cases, pregnancy resulted and eight young were produced—all true rabbits, not hybrids.

The authors conclude that in their experience it is impossible to get hares to mate naturally with rabbits, and even if this were to occur under exceptional circumstances, no hybrid offspring would result because of the evident inability of the rabbit egg to be fertilized by hare sperm.

We may accordingly relegate the hare-rabbit to the limbo of zoological myths, along with the unicorn and the sea serpent.

Those who regard evolution as a consequence of hybridity alone will need to look elsewhere for proof.

W. E. CASTLE

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HAS PARTHENOGENESIS BEEN CONFUSED WITH HERMAPHRODITISM IN CUCURBITA?

IN a recent paper by A. C. and A. L. Hagedoorn[1] experimental evidence is given that tends to show that parthenogenesis occurs in the cucurbita.

Since Lotsy's criticism in 1919 of a former paper by these authors we have been running check experiments, the results of which do not support the European workers.

Work was done on stimulation of stigmas in 1922. This consisted of cutting and crushing of tissues and placing foreign pollen on the stigmas. No fruit development occurred.

During the past season 301 female buds were tied and bagged as a double check against insect invasion. No fruits developed. As 57.9 per cent., 1,005 out of 1,733, of the blossoms pollinated matured and the fruits were harvested, it can be concluded that in the strains of *Cucurbita pepo* used parthenogenesis does not occur.

In a few open pollinated hermaphroditic fruits maturity occurred where the fruit stem was short.

Some of the figures in the Hagedoorns' paper show the characteristic receptacle cracks and navels of hermaphroditic fruits.

As these figures show individuals belonging to the genera *C. pepo* and *C. maxima* being crossed and fertile offspring resulting, the results seem to be directly opposite to those obtained by workers in this country.

If, however, they bagged a hermaphroditic fruit of *C. maxima* their results may be explained on the basis of being direct pure line descendants rather than descendants of a generic cross that has constantly been proven sterile. Figures 1 and 7 in their paper suggest such a condition.

[1] Hagedoorn, A. C. and A. L., 1924, "Parthenogenesis in Cucurbita." *Zeit. Ind. Abst. u. Vererbungelehre,* pp. 186–213, figs. 1–8.

Russell, p. 1924, "Identification of the commonly cultivated species of cucurbita by means of seed characters." *Jour. Wash. Ac. Sci.,* pp. 265–269, figure 1.

Further work will be carried on here as a check on the results of workers on the cucurbita that have been published to date.

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EGG-LAYING IN TWO SPECIES OF PLANORBIS

FIVE individuals of *Planorbis corneus* L., and twenty-one of *P. trivolvis* Say,[1] were kept in a large balanced aquarium, except during the periods of experimentation described below, during the winter and spring of 1922–1923, and observations were made upon the number of eggs laid, and the periodicity of the process.

Among the *P. corneus* specimens egg-laying began on December 30 and continued to February 5. During this time 20 masses of eggs were deposited upon the glass walls of the aquarium and five upon the stems of water plants. No eggs were found elsewhere, and in all cases the eggs were laid at night. The number of eggs in the masses varied from 13 to 27, and the total number of eggs in all the masses was 456. As egg-laying continued the number of eggs per mass steadily decreased. The data give the following averages: 18.2 eggs per mass; 5 masses or 91.2 eggs per individual. Soon after discovery the eggs were isolated and proper precautions taken to allow the normal development of the embryos. Ninety-two per cent. of the eggs succeeded in hatching from the capsules, but after that stage the mortality from bacterial and fungal infection was so high that only 21 individuals survived through metamorphosis.

The 21 *P. trivolvis* specimens began egg-laying on April 12 and continued until April 23. A total of 872 eggs were laid; 32 masses on the glass walls of the aquarium and 3 on leaves of water plants. All of them were deposited during the night, and as egg-laying continued the number of eggs per mass decreased. The averages were as follows: 21.3 eggs per mass; 2 masses or 41.5 eggs per individual. No precautions were taken to allow these eggs to develop.

It appears therefore that *P. corneus* lays over twice as many masses and eggs per individual as does *P. trivolvis;* although the latter lays more eggs per mass than does *P. corneus.* It is further evident that both species deposit the majority of their eggs

[1] The writer is indebted to Bryant Walker for identifying these species.

on the glass walls of the aquarium, that they both lay their eggs exclusively at night, and that as egg-laying continues the number of eggs per mass decreases. The number of eggs laid by other species of snails varies considerably. Baker[2] (p. 271) describes an individual of *Limnaea caperata* which deposited 5 masses between April 16 and 19, containing 45, 42, 42, 35 and 28, respectively, totaling 192 eggs, or an average of 38.2 per mass. In the case of Physa gyrina, Baker says (p. 314) that 15 specimens deposited 14 masses from April 23 to 24, three of these containing 130, 160 and 200 eggs per mass, or an average of 163.3. No indication is given of the chronology of laying of the three masses. It is further stated (p. 360) that *Valvato tricarinata* "lays from 10–30 eggs," with the inference that these figures represent the total number of eggs laid. Clapp[3] reports the number of eggs in masses from *Ancylus fuscus* as 7 to 8 at first, but that in the capsules deposited later the number decreases steadily down to 1. Anderson,[4] who studied the breeding habits of three species of Bullinus under controlled experimental conditions, gives the following data: Number of eggs per mass, 1 to 11; maximum number of eggs per individual, 19; total number of eggs, 138, deposited in 35 masses by 17 animals. His figures indicate that the number of eggs per mass did not increase as egg-laying continued, and that the length of the egg-laying period varied greatly among the different animals. Although he noted that copulation occurred during the daytime as well as at night, he does not say when the eggs were deposited.

The fact that *P. corneus* and *P. trivolvis* laid their eggs only during the night raised the question as to whether or not the periodicity was controlled directly by the environmental conditions of temperature, illumination and carbon dioxide content of the water. During the night the temperature decreased five degrees or more, light was absent and the CO_2 content of the water increased, due to the cessation of photosynthesis in the water plants. The three factors, therefore, exhibited a daily

[2] Baker, F. C., 1902, The Mollusca of the Chicago area. Bull. no. 3 of the Natural History Survey, Chicago Acad. Sciences.

[3] Clapp, W. F., 1921, Eggs and young of the river limpet, Ancylus fuscus C. B. Adams. Occasional papers of the Boston Soc. Nat. Hist., vol. 5, p. 5.

[4] Anderson, C. W., 1923, Enquete et recherches sur la bilharziose en Tunisie. Arch. Institut Pasteur de Tunis., T. xii, no. 1. (For this reference and for quotations from it, the writer is indebted to Dr. W. J. Crozier.)

rhythm of maximum and minimum. To determine any relations between this rhythm and egg-laying, 24 individuals were isolated in separate jars. Twelve were kept in continuous darkness, and 12 in continuous light, by using a 150-watt Mazda lamp, 30 cm. above the jars, and separated from them by a water heat screen. Records of temperature showed the same variations during the day and night as those of the large aquarium. At the end of 96 hours, after which 18 egg masses had been deposited, the experiment was discontinued. All of the eggs had been laid at night, 10 masses by the "dark" animals, and 8 by the "light" ones. In the second experiment all of the animals were kept in continuous light during the night and in darkness during the day for 96 hours during which time the temperature varied less than 2° C. Under these conditions 14 masses were deposited, all of them during the night, even though the normal environmental conditions of day and night had been reversed. Further tests were impossible because of the cessation of egg-laying.

It is concluded that egg-laying in these species is controlled by a periodicity which must be regulated by internal processes, and not directly by the external environmental conditions of light, temperature and CO_2. The possibility of changing the rhythm by reversing the normal conditions over a long period of time preceding egg-laying is of course not precluded. Duirnal periodicity in the deposition of eggs in other animals is a common occurrence. For Gasteropoda, however, the references are few. Eales[5] (p. 71) says that for *Aplysia punctata*, the Sea Hare, "deposition usually takes place at night in the aquarium," and Clapp[3] reports (p. 5) that *Ancylus fuscus* laid all their eggs at night. This sort of periodicity then must belong to a different category from that found in Copepoda, whose daily depth migrations seems to depend directly upon the conditions of illumination, temperature and CO_2 content of the water,[6] and from that found in other animals due to similar environmental changes.

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[5] Eales, N. B., 1921, Aplysia. Liverpool Marine Biological Memoirs, no. 24. Liverpool.

[6] Loeb, J., 1905, Studies in general physiology, Pt. 1, p. 282. Chicago.

A HISTOLOGICAL METHOD FOR THE STUDY OF PIGMENTATION IN MAMMALIAN EPITHELIUM

THE object of the following note is to record a histological method for the determination of the location of pigment in the skin of rodents.

The material used in this work was epithelium from the tails of black mice. Some of these mice were uniformly black in color, while others had white spotting on their tail. Separate discussion of these two varieties is not necessary for the purpose of this paper.

According to many authors, pigment is situated directly beneath the epidermis. Bohm, Davidoff and Huber (page 404) say that pigment in the skin can best be studied in unstained sections. Guyer (page 49) gives methods for preparing skin for histological study.

Following Guyer's method in the case of pigmented skin proved inadequate, for although the cellular detail was good, there was no trace of pigment granules. An attempt was made to follow out Bohm, Davidoff and Huber's suggestions of using unstained preparations, but insufficient detail resulted, although there were visible pigmented areas.

After some experimentation, the following method proved to be successful and is recorded in the hope that it may be of service. The mouse was killed, a superficial incision was made around the tail, about three fourths of an inch from the tip. By gently twisting and pulling, the epithelial tissue came off as a sheath leaving behind the skeletal and connective tissue. The sheath was then split longitudinally, spread out flat and bound in position by fine silk thread to a flat piece of cork about one inch long by one half inch wide and one eighth of an inch thick. Thumb tacks stuck into the bottom of the cork served to keep it in an upright position immersed in the fixing solution.

The following method of fixing, sectioning and staining was found to be the most successful:

Fixation in Bouin's for three hours; three washings of 70 per cent. alcohol for 20 minutes each; 85 per cent. alcohol for 20 minutes; 95 per cent. alcohol for 20 minutes; absolute alcohol for 45 minutes; xylol for 30 minutes, and 45 degree melting point paraffin in an oven constant at 50 degrees, for one hour. Then the specimens were imbedded and sectioned at seven micra

in thickness. They were stained in Delafield's hematoxylin for five, ten or fifteen seconds. Each of these gave excellent differentiation of pigment and cell structure. The five second slides were, on the whole, the best. Longer staining showed clearly that the pigment became either masked or destroyed.

It is not known how rapidly the sections will fade, but at three months after preparation, they are still entirely satisfactory for general study.

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